Dr. Bernard Mallet

10TH STEERING COMMITTEE MEETING ON ICSB/CIRAD-FORET COLLABORATIVE PROGRAMMES

25-30 APRIL 1999
RBJ CONFERENCE ROOM, TAWAU

REVISED PROGRAMME (23 APR '99)

10TH STEERING COMMITTEE MEETING ON ICSB/CIRAD-FORET COLLABORATIVE PROGRAMME, 25-30 APRIL 1999, RBJ CONFERENCE ROOM, TAWAU, SABAH

DATE	TIME	ACTIVITIES
25 April 1999 *(Sunday)*		Arrival of participants in Tawau *Night in Marco Polo Hotel*
26th April 1999 *(Monday)*	0800-1000 1030-1700 1930	Visit Plant Biotech Lab 10th Steering Committee Meeting Welcoming dinner @ Maxim Restaurant *Night in Marco Polo Hotel*
27th April 1999 *(Tuesday)*	0800-1700	10th Steering Committee Meeting continues *Night in Marco Polo Hotel*
28th April 1999 *(Wednesday)*	0730 1200 1300 1700	Depart for Taliwas by road Visit Taliwas Lunch at Danum Valley Field Centre Visit FACE Foundation Project Return to Tawau *Night in Marco Polo Hotel*
29th – 30th April 1999 *(Thursday-Friday)*		Visit LFC *Night in LFC*
1st May 1999 *(Saturday)*		Departure

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10TH STEERING COMMITTEE MEETING ON ICSB/CIRAD-FORET COLLABORATIVE PROGRAMME, 25-30 APRIL 1999, RBJ CONFERENCE ROOM, TAWAU, SABAH

AGENDA

1. Welcoming remarks by the Group Manager, Forestry Division
2. Confirmation of Minutes of 9th Steering Committee Meeting
3. Matters arising
4. Activity Progress Report
   - 4.1 Plant Improvement and Seed Production (PISP)
   - 4.2 Plant Biotechnology Laboratory (PBL)
5. Technical Notes/Reports
6. Any Other Business

MINUTES OF 9TH STEERING COMMITTEE MEETING, ICSB-CIRAD-FORET COLLABORATIVE PROGRAMME, 2-3 APRIL 1998, TAWAU, SABAH

Present

ICSB

Awang Mohdar Hamzani	Chairman
Chan Hing Hon	
Charles Garcia	
Dr. Doreen Goh	
David Alloysius	
Andrew Garcia	
Ag. Rahim Ag. Ali	
Esther Li	
Soo Sau Mee	
Azlan Budi	
James Rubinsin Kotulai	
Gideon P. Joitol	
Bolgen Majingin	
Yusrin Yusof	
Abd. Wahab Latip	
Wilter Malandi	
Hanna Moo	
Jikos Gidiman	
Frederica Mojilis	Secretary

CIRAD-Forêt

Dr. Bernard Mallet
Dr. Philippe Vigneron
Dr. Roberto Bacilieri
Dr. Antoine Galiana
Wilfred Schueller
Kevin Pouet

1. WELCOMING REMARKS

The Chairman welcomed Dr. Bernard Mallet who is the Head of Forest Trees and Plantation Programme, Dr. Philippe Vigneron, Wilfred Schueller, Junior Scientist and Kevin Pouet, all from CIRAD-Forêt.

Mr. Patrick Durand was not able to participate in the meeting as he was on his way back to France due to the closing down of CIRAD office in Singapore. Mr. Tang Hon Tat who had not failed to attend all the 8 steering committee meetings was also absent due to both economic and logistics reasons.

The Chairman expressed his sincere gratitude to all scientists from CIRAD-Forêt for their hardwork and dedication to the project which is now into the 9th year.

He further commented that timber as a commodity is very much affected in the almost collapsed market situation and ICSB is facing stiff competition from the neighbouring countries. In line with the economic downturn, there is a need for ICSB to re-orientate its policy on LFC. He mentioned that there shall be no major planting but emphasis shall be stressed on the existing plantation. Effort should be focused to proper silvicultural techniques or applied research on how well the present stands can be improved.

The Chairman informed the meeting that ICSB has already invested RM30 million into the LFC plantation project. Sabah Softwoods Sdn. Bhd. has become a wholly-owned subsidiary of ICSB and would require research support in its industrial plantation programme. RBJ, through the

PISP/PBL programme have already started to produce *A. crassicarpa* and acacia hybrids through tissue culture. IKEA may also come in to rehabilitate degraded forest lands and this again will provide a major boost to LFC.

2. CONFIRMATION OF MINUTES OF 8TH STEERING COMMITTEE MEETING

Confirmation of Minutes of the previous meeting was proposed by Chan Hing Hon and seconded by David Alloysius.

3. MATTERS ARISING

3.1 Vegetative Propagation Techniques

The meeting was informed that there were still a lot of teak plantlets at Taliwas.

For information only

3.2 Reproductive Biology Study of Rattan

DA informed the meeting that his article on controlled pollination study is in the process of being published in CIRAD-Forêt bulletin or Journal of Tropical Science.

For information only

3.3 SSSB as a potential buyer of tissue cultured plantlets

CHH informed the meeting that SSSB has signed an agreement with RBJ to produce 20,000 plantlets of *Acacia crassicarpa* or acacia hybrids within one year. So far they have collected 40 clones of hybrids.

For information only

4. ACTIVITY PROGRESS REPORT

4.1 Plant Improvement and Seed Production (PISP)

The detailed report on the following research studies can be found in the PISP 1997 progress report.

4.1.1 Rattan

DA presented the progress on rattan research.

4.1.1.1 Establishment of field trials

Some new trials were established at the end of 1997 to transfer material collected in 1996 to the field:-

Species	Type of Planting	Plot number	No. of progeny	No.of seedling	Date planted
C. ornatus	Progeny trial	COB2	30	270	Dec 1997
C. subinermis	Resource stand	R6	11	227	Dec 1997
C. manan	Resource stand	R7	2	120	Dec 1997
C. optimus	Resource stand	R2	5	150	Dec 1997

4.1.1.2 Assessment of established trials

The rattan annual growth assessment for 1997 was delayed due to some other work that was considered at higher priority such as the establishment of teak trials that needed to be completed in time.

4.1.1.3 Reproductive biology of rattans

First attempt of control pollination (CP) in *C. manan* has been made in March 1996. However, experiment was terminated due to the destruction of the flowers under observation because of fallen tree branches. In January 1997, another experiment was initiated in order to conclude the previous experiment. The objectives of the experiment were:-

i. To develop a CP technique for *C. manan*.
ii. To evaluate the suitability of different type of materials as pollination bag.
iii. To test simple pollen storing methods in CP of *C. manan*.

The time frame for the experiment is February 1997 to June 1998.

Some preliminary conclusions derived from this early result are :-

i. Hand pollination is possible for *C. manan*
ii. Stored pollen at 4°C for 22 hours could be used for pollination of *C. manan*.
iii. Plastic is not a good material for the pollination bag.

The problems with CP are :-

i. Long maturation period (15-16 months).
ii. Time consuming.
iii. Possibility of agamospermy and self-pollination.

4.1.2 Trees

RB presented the progress on the tree species. The results of the various studies can be found in the PISP progress report for 1997.

4.1.2.1 Industrial species

1. Acacias

a. Seed collection

There was no seed collection made on the *A. mangium* seed orchards in 1997 mainly because a large stock was already available and there was no demand from buyers.

b. Field trial of seedling vs micro-propagated *A. mangium*

This experiment had the principal objective to evaluate two propagation methods for Acacia mangium, from seeds and from tissue culture. The main finding was that there was no difference between seedlings and micro-cuttings of clone n. 5, either for survival, diameter and height or stem form. Also the hypothesis of a better uniformity of clonal material could not be confirmed.

c. Study of heart rot disease on Acacia mangium

A core sampling of 3 Acacia trials (2 *A. mangium* and one *A. auriculiformis*) was carried out in 1996 by the PISP to study the incidence of the heart rot disease on Acacias. The main results were:-

A. mangium was more sensitive to the disease than *A. auriculiformis*. However, at age 6, the portion of attacked wood was not very important and would not really affect the production of paper pulp if this was the final wood product. The disease may further progress with the age of the trees.
No genetic effect involving a resistance to the disease was detected. By contrast a block effect was present, suggesting some environmental conditions favouring the disease.

ii. *Octomeles sumatrana* (Binuang)

Two silviculture trials of Binuang were planned in 1995 at KM13, Taliwas. As the effects of the treatments were evident, both the trials were declared closed in 1997. The data was sent to CIRAD-Forêt, FRANCE where contour maps would be prepared for both diameter and height. The map would be prepared for both diameter and height. The map received still had some problems but upon resolving the problems, block partition based on the existing stand condition can be indicated and thinning can then be carried out before end of 1998.

iii. High-value species

For the high value timber species, the PISP activities focused mainly on three species, namely *Tectona grandis* (Teak), *Khaya ivorensis* and *Xylia xylocarpa.*

a. Teak

The commercial production of teak was handed over to the Nursery Unit in May 1997. However, the acclimatization of plantlets from PBL is still the PISP responsibility. About 9,000 macro and micro-cuttings of teak were sold from Luasong in 1997. Another 25,000 were used for establishment of trials in Taliwas and Luasong.

b. *Khaya ivorensis*

A pilot plantation of this species was established in one of the compartment in Luasong plantation as follows:-

In October, 32 kg of seeds were received from Ivory Coast through CIRAD-Forêt.
20 kg were germinated upon arrival and the germination was quite good (50%).
The remaining seedlots were first stored at 4°C and germinated later. However, the passage from 4°C (CIRAD cold room, France) to open air (airport, quarantine) and then to 4°C again (PISP's cool room) probably damaged the seeds as the germination of the last batch was very poor.

The 32 kg of seeds included 19 progenies plus a bulk of the two best African provenances (Mopri and Yapro). The Plantation Unit planted a controlled

mixture of the 19 progenies and of the bulk as a seed stands for the future needs of ICSB. A number of trees from the 19 families and from the bulk were also sent to the PISP nursery for vegetative propagation.

c. *Xylia xylocarpa*

In the line planted plot, a shade adjustment was done in July 1997 in order to liberate the Xylia trees. It is not possible to conclude on the effect of liberation, as a proper control does not exist.

One of the main tasks for PISP in 1997 was to develop VP techniques for *K. ivorensis* and *Xylia xylocarpa*. The experiments in 1997 concluded that:-

Cuttings can be produced from coppices of felled trees
The size of the cuttings is between 4-6 cm in length
In the present conditions, rooting takes between one to two cuttings for both species
The average rooting was 48% for *K. ivorensis* and 44% *X. xylocarpa*

4.2 Plant Biotechnology Laboratory (PBL)

Dr. Goh/Dr. Galiana presented the progress report on the main activities of the PBL in 1997. Results of the studies can be found in the PBL progress report for 1997.

The main activities of the lab for 1997 were R&D on Teak, Acacia and Rattan (large cane species) and also commercialization.

4.2.1 Teak

4.2.1.1 Teak micro-propagation

Teak micro-propagation is very well established in the lab. About 75,000 micro-shoots were produced and transferred to the nursery.

4.2.1.2 Teak meristem culture

The introduction of shoot apical meristems of teak, ranging from 0.1mm to 0.2mm has been allowed to be introduced of true meristem cultures of mature teak genotypes.

The obvious advantages for mass propagation of teak through meristem culture are:-

i. Successfully introducing selected clones in vitro without contaminations;
ii. Eradication of possible endogenous contaminants;
iii. Stimulation – rejuvenation – of the potential for true-to-type cloning;
iv. Germplasm exchanges, etc.

This technology was initiated and improved by Dr. Olivier Monteuuis and was successfully carried out and to date, five meristems consisting of 1,3, 5, 7 and 8 have been introduced and multiplied in vitro.

To-date, several hundreds plantlets arising from only one meristem (M-1) from Solomon Islands have been sent to LFC for acclimatization and subsequently used in the provenance/progeny field trial.

In addition to M-1, several hundred plantlets from meristems of stock plants of Solomon Island origin in the nursery have been multiplied in the lab.

4.2.2 Acacia

Micro-propagation of Acacia species was undertaken in view of the large-scale plantation programme in Sabah and South East Asia for pulp production.

ICSB's agreement with SSSB to produce in-vitro production of 20,000 plantlets *of Acacia mangium* and *A. crassicarpa* is also relevant. The main advantage of Acacia micro-propagation was less time consumed for rejuvenation while the drawback was that it was not a cost-effective method for producing low-value timber species.

Until last year, the micro-propagation experiments on acacias were only performed on *A. mangium* where juvenile and mature selected materials were compared. Some experiments of introduction through nodal culture or micro grafting were also shown to be successful on putative hybrid material (clone no. 1 from Taliwas). The same multiplication media were used for both *A. mangium* and Acacia putative hybrids in those previous experiments. However, initial studies performed in CIRAD-Montpellier in 1995 showed that the Acacia hybrids required specific media for multiplication as were found opposite responses from the two species when submitted to different hormone combinations.

In acacias, the micro-propagation process consists of transferring the plantlets onto successive multiplication sub-cultures in a specific medium before transferring all the multiplicated plantlets on a rooting medium at the end of the production cycle.

4.2.3 Rattan

Research activities on the three selected Calamus spp., *C. manan, C. merrillii* and *C. subinermis*, were focused on the multiplication through axillary (or adventitious) budding of tissue culture germinated seedlings, rejuvenation or multiplication of explants from materials collected in the nursery, and regeneration through the process of somatic embryogenesis.

Using the process of somatic embryogenesis, for *C. manan*, most explants formed calli but did not differentiate into definite proembryogenic structures. For *C. subinermis*, the most responsive explants appeared to be that of roots. *C. merrillii* demonstrated the most prospect for regeneration using the process of somatic embryogenesis. Explants using zygotic embryos and young leaves from in-vitro grown seedlings were highly responsive to the growth regulator picloram following 6-8 weeks of inoculation.

4.3 Commercialization

A feasibility study to commercialize teak production was initiated in 1997.

4.3.1 Sale of plantlets

The commercial production and sale of teak plantlets took off in late November 1997. Origins used in the sales comprised of Solomon Island and Perlis.

For the period from November to December 1998, 27,000 plantlets were sold and the total proceeds was RM62,500.00. The plantlets were sold to Maju Aik Sdn. Bhd. and RISDA.

4.3.2 Service contracts

The PBL also undertook a new task on providing services for the micro-propagation of selected clonal materials for interested customers. Presently, micro-propagation services are provided only on the species currently studied in the PBL, which are teak and acacia species.

4.3.3 ICSB-FRIM

FRIM had signed a 5-year service contract with ICSB on 1st January 1998 for the purpose of micro-propagating their selected clones.

4.3.4 ICSB-SSSB

Prior to the service agreement between the two parties, ICSB management in November 1997 requested a feasibility study on mass propagation of Acacia spp. This study was prepared and submitted based on findings/observations from previous experiments in the PBL and other researchers. A presentation of the prospects and potential setbacks of mass production of Acacia spp. was made by PBL scientists to the Executive Director of Operations of SSSB on 8th January 1998. A second service contract was then made between SSSB and ICSB on 15th January 1998 for the micro-propagation of selected clonal materials of *A. crassicarpa* and Acacia hybrids from the SSSB's area. 20,000 plantlets of these species were requested in this agreement and the first delivery of the plantlets is expected in the first quarter of 1999.

4.4 Forestry GIS

4.4.1 Spatial data capture

76% of on the YS concession area have been captured.

4.4.2 Data dictionary

A new data dictionary has been made using freeform database which support expandable items. It is based on a freeware program called Vault.

4.4.3 SML programming for Arc Info

A simple SML program called Arc Editor was created to facilitate editing.

4.4.4 GIS hardware and software

A new inkjet plotter, HP Designjet 750 Plus was acquired in May 97 and an A3 sized printer, Canon BubbleJet BJC4550 in April 1997.

A networking has been set up to link the 4 units of computers in the Cartography/GIS Unit in October 1997 to enhance data sharing. The Pentium 100mhz computer has been replaced by Pentium Pro 200 Mhz and it is used as the server for the network. The new version of Arcview is also installed in the upgraded computer.

For Luasong, ArcView 3.0a was acquired from ESRI and installed on one of the computer in the computer room. Both ArcView and PC Arc/Info software in the GIS unit have been upgraded to the latest version.

4.4.5 Future development

In view of the requirement by Forestry Department to submit a Forest Management Plan (FMP) for all its FMUs before the year 2000, the GIS Unit can play a major role in preparing the data set for the FMP especially in obtaining the slope classification and stratification of forest type. It is therefore proposed that 2 new set of computers and one set of PC Arc/Info and Arcview each with the 3D Analyst extension.

Training will also be conducted to selected staff on aerial photo interpretation and stratification of forest type. Stratification works will also be done covering the whole concession area as required for preparation of the FMP. The forest type will then be captured in GIS for further analysis and final map preparation.

The GIS Unit is also planning to create a homepage to disseminate information and project a better understanding of the GIS activities.

5. CONCLUDING REMARKS

The Chairman mentioned that there was lack of synergism between research and operation. The research results need to be synchronized with the field operations. He further reiterated that the research results need to be carried out in the field, monitored by a team comprising a key person from research, operation and headquarters.

Mr. Mallet was very impressed with the quality of research being carried out and he also agreed that the research results need to be applied in the field.

The Chairman thanked all present for the meeting especially the scientists who put a lot of effort in the research activities.

There being no other business, the meeting was adjourned at 9.15 a.m., 3rd April 1998.

Recorded by:

(Frederica Mojilis)
SECRETARY

Confirmed by:

(Awang Mohdar Hamzani)
CHAIRMAN

AGENDA 4.1

PLANT IMPROVEMENT AND SEED PRODUCTION (PISP)

PLANT IMPROVEMENT & SEED PRODUCTION

PROJECT

ANNUAL REPORT FOR 1998

Activity Report for the Steering Committee Meeting n° 10

(April 1999)

ICSB / CIRAD-Forêt

INTRODUCTION

The PISP Project was started in 1989 after the signing of the first Memorandum of Understanding between ICSB and CIRAD-Forêt. In 1997, PISP has entered the third phase of the collaboration after expiry of the second phase (July 1992 to July 1997). The objectives of PISP are as follow:

Short term objectives

To develop a plant improvement strategy of rattans, high-value timber species, and industrial timber species

To develop a seed/planting material production programme for rattans, high-value timber species, and industrial timber species to meet the seed and other planting material requirements of ICSB

To develop the technical capability in plant improvement and seed/other planting material production of ICSB

Long term objectives

To develop commercial seed production stands/orchards, on a joint venture basis, to meet the seed/other planting material requirements of state, national or international institutions

To improve the technical capability at LFC to the level necessary to be consistent with ICSB's objective of LFC into a centre of excellence for tropical forestry management, development and research, and

To enable ICSB/CIRAD-Forêt to undertake expertise in the relevant fields if the opportunities arise.

Personnel

There was a decrease in the number of staff compared to the number before the last Steering Committee Meeting. Forest Officer Wilter Malandi resigned in April 1998 after more than a year with PISP. Forest Ranger Arbani Mamang was transferred to the INIKEA Project. The number of casual staff decreased by 5. The French volunteer scientist, Wilfrid Schueller, who replaced the former volunteer that left in May 1997, reported for duty in April 1998. The staff line-up as end of March 1999 is as follows:

Position	ICSB	CIRAD-Forêt
Senior Scientist		
Junior Scientist		
Senior Forest Ranger		
Forest Ranger	3	
Casual labourer	29	
TOTAL	34	2

1. RATTANS

The activity on rattans has been slowed down following to decision taken during the last Steering Committee Meeting (1998). No collection of germplasm or growth assessment were made in 1998. For genetic improvement, several trials were established using materials collected in 1997. Routine up-keep and maintenance works were done in all established trials totaling about 70 ha. Annual assessments were made in most of the established silviculture trial plots in the plantation.

1.1 GENETIC IMPROVEMENT

1.1.1 Establishment of field trials

Progeny trial/resource stand

Two resource stands were planted using progenies collected from Pulau Banggi. In our routine, a resource stand is established whenever an interesting genetic material is available in low quantity that does not allow the establishment of a true genetic trial. Summaries of the trials are presented in Appendices 1 to 4.

Table 1. Rattan trials established in 1998

Species	Type of planting	Plot number	No. of seedling	Planted date
Calamus ornatus	Resource stand	R2	90	July 98
C. subinermis	Resource stand	R7	321	July 98

Living collection

Two more species were planted in the Luasong's Wild Rattan Conservation Area, the area where species with less or no commercial value are planted. The total number of species as end of March 1999 is 25.

Table 2. Rattan species planted in Wild Rattan Conservation Area in 1998

Species	Origin	No. of seedling	Planted date
Daemonorops longipes	Pulau Banggi		July 98
Plectocomia mulleri	Pulau Banggi	11	July 98

1.2 SILVICULTURE

1.2.1 Assessments

The PISP, with the assistance of the Silviculture Unit (ICSB) has established in the past a number of silviculture trials for rattans in the field. In 1998, the following trials have been assessed (Table3):

Table 3. Silviculturc trials for rattan asscsscd in 1998

Name of the trial	Species	Date of planting	N. of assessment
Comparison of container size and plantation method (bare root or with soil)	*C. subinermis*	Jan 1997	III (Aug 1998)
Comparison of container size	*C. caesius*	Nov 1996	III (Aug 1998)
Fertiliser trial	*C. subinermis*	Feb 1997	III (Aug 1998)
Size of the plants at plantation	*C. caesius*	Nov 1996	III (May 1998)
Growth pattern	*C. caesius*	Nov 1996	XI (Aug 1998)
Temporary growth/environment plots	*C. subinermis*	Jun 1998	I (June 1998)
Shade adjustment trial	*C. subinermis*	Jul 1996	III (May 1998)
Yield plots	*C. subinermis*	1990-1991	VII (Jun 1998)

1.2.2 Analysis

Light and competition

In 1998, PISP has received a student from the National Agronomy Engineering School of France, M. Kevin Pouet, on a period of six months. M. Pouet participated in the measurements of the shade adjustment trial and of the temporary growth sampling points in a *C. subinermis* planting. He studied and improved the method of measuring the light in the forest with the LICOR sensors, and applied his findings to the measurement of light in the above trial and temporary plots. His work gave origin to a thesis entitled: "Characterisation of the light and competition environment in a line planting of *Calamus subinermis* under logged-over forest in Sabah".

Yield plots

The measurements on the yield plots of *C. caesius* (1997) and *C. subinermis* (1998) have been analysed in 1998, with a view to calculate the percentage of mature cane and revenue per hectare. The results are attached in the Appendices (5 and 6) to this report. Generally, the revenue figures are low, pointing at the following causes: 1. Low rattan market price; 2. Poor and heterogeneous growth; 3. Pest attacks, especially on *C. caesius* (shoot borers) and on *C. subinermis* (elephants and other mammals).

Silviculture trials

A paper entitled "Silviculture of Rattans under Logged-over Forest" was presented at an international meeting on rattans in Kuala Lumpur. The paper summarised the results of the measurements of the silviculture trials from 1991 to 1998.

The data of the 1998 assessments have not been published yet. Globally, the results of this last assessment did not bring any new enlightenment compared to the previous analysis made during the former assessments. It seems that the differences due to the treatments (fertiliser, container and plant size, shade adjustment, plantation method) keep following the schemes already detected (Bacilieri *et al.* in press)

2. TREES

A list of all trials planted with trees in Luasong and Taliwas since 1990 is provided in Appendix 7. The total net area for the tree trials is 58 ha. To this it must be added minor trials, demoplots and hedge plant parks that were established occasionally both in Taliwas and Luasong.

2.1 ACACIAS

2.1.1 Growth assessment

Annual assessments of all Acacias plots except *A. aulococarpa* were made as usual. *Acacia mangium* is still the best performer followed by *A. crassicarpa*. The mortality in *A. crassicarpa* plots was somehow high (9%) compared to less than 1% in other Acacias plots. The cause of the mortality is probably termite attack.

Table 4. Average values for the several Acacia trials at the 19998 assessment.

Species & plot	Age (yrs)	No. of Living trees	Mean height (m)	Mean diameter (cm)	HMAI (m/yr)	DMAI (cm/yr)
Acacia mangium						
(PNG)	8.8	134	29.2 (2.7)	33.0 (5.8)	3.3	3.8
SSO1	8.8	165	28.9 (2.9)	32.0 (5.9)	3.3	3.7
SSO2	8.8	137	27.1 (2.8)	31.4 (4.6)	3.1	3.6
SSO3						
Average			**28.4**	**32.1**	**3.2**	**3.7**
A. mangium (QLD)						
SSO1	8.6	157	26.0 (3.4)	30.6 (7.7)	3.0	3.6
SSO2	8.6	153	28.1 (2.8)	33.8 (5.8)	3.3	3.9
SSO3	8.6	307	26.5 (3.7)	29.3 (6.8)	3.1	3.4
Average			**27.6**	**31.7**	**3.2**	**3.7**
A. auriculiformis						
SSO1	8.8	207	24.8 (3.1)	25.9 (5.6)	2.8	3.0
SSO2	8.8	251	25.1 (2.5)	24.8 (6.1)	2.9	2.8
SSO3	8.8	186	24.5 (2.4)	26.6 (6.4)	2.8	3.0
Average			**24.8**	**25.8**	**2.8**	**2.9**
A. crassicarpa						
SSO1	8.7	102	24.7 (2.7)	31.3 (5.2)	2.8	3.6
SSO2	8.7	119	24.6 (2.6)	30.9 (5.8)	2.8	3.6
SSO3	8 7	101	26.5 (2.2)	31.4 (4.9)	3.1	3.6
Average			**25.3**	**31.2**	**2.9**	**3.6**

Note: Value in parenthesis indicates standard deviation

2.1.2. Seed collection

Ten kilograms of seeds were collected from the *A. crassicarpa* seed orchards in March and despatched to the Sabah Forest Industries (SFI). A new collection is planned in 1999 to collect more seeds of this species. Fruiting of *A. crassicarpa* in Luasong is scarce and the seed processing is relatively difficult compared to *A. mangium*. The current stock of *A. mangium* seeds in PISP's storage room is 98 kg.

2.1.3. Genetic improvement – Sabah Softwood. Sdn Bhd.

Following new contacts established between ICSB and Sabah Softwood Sdn. Bhd. (SSSB), and a contract passed between SSSB and the Plant Biotech. Laboratory (PBL) for the clonal *in vitro* production of *Acacia crassicarpa* and Acacia hybrids plants, an analysis of the genetic trials of

these two "species" in Brumas and Luasong has been undertaken. The objective of this work was to accompany the propagation work of the PBL with an accurate analysis of the genetic value of the clones.

Acacia mangium x auriculiformis hybrids

The trial Ah18c planted in Brumas includes 144 clones produced from seedlings collected in a bi-specific (*A. mangium* and *A. auriculiformis*) seed orchard. In 1992, the seeds collected in the bi-specific seed orchard were germinated, and a number of putative hybrid seedlings were selected based on their intermediate morphology. These seedlings were propagated by cutting, and 144 clones were established in the field in September 1993 in a single-tree randomised complete block design with 6 repetitions. Being a single-tree design, each repetition included only one copy of a given clone, so that over all six repetitions there were only 6 copies per clone. The trial has been assessed every year since, but we only studied the assessment of 1998 (even if the other assessments were useful to trace the history of the trees).

In addition to the existing data, in June 1998 we went to Brumas to assess the form of the stem section, which in the trial is sometime very far from the circular. At a first sight, it appears that mortality, performance of the trees and shape of the stem section are correlated: we hope to be able to use this latter as an early predictor of growth.

The trial has been affected by a certain amount of mortality (42%), which reduced again the number of trees per treatment. Due to the low number of trees within treatment, the statistical analysis of the 1998 assessment did not yielded any significant differences among clones. Statistical tests being not significant, selection can either focus on the trees that survived well (for example clones n. 41, 78, 131, 60, 8, 68), or on those with an acceptable survival and with the maximum volume (clone n. 116, 72, 37, 46, 51, 79). Overall, the best clone seem to be n. 41, collected on a maternal parent tree *A. auriculiformis* [AA7d]; in the trial, clone n. 41 has 100% survival and is only seventh (out of 144) in the ranking for volume (Appendix 8).

Acacia crassicarpa

Several progeny trials have been established in the past with the same genetic material of *Acacia crassicarpa*, three in Luasong (SSO1, SS02 and SSO3) and one in Brumas (AC20a). In order to study the genetics of these families in different environments, to assess the genetic*environment interaction and to prepare a selection that is valid over different sites, we carried out a joint analysis of SSO1, SSO2 and AC20a. The results are joined in Appendix 9 to this report.

2.1.4. Field trial of seedling versus micro-propagated clones of *A. mangium* clone n. 5

This trial, established in Nov 1, 1996 using both *in vitro* plantlets and open pollinated seedlings from clone n. 5, has been assessed once in 1998 and once in February 1999. The data analysis is reported in the Appendix 10 attached to this report. The main result is that there is no significant difference between micro-propagated plants (clones) and the seedlings of clone n. 5, neither for growth nor for the homogeneity. A slight difference, not significant, in growth was in favour of seedlings.

2.1.5. Vegetative propagation - Rooting and coppicing experiments

Several experiments were carried out in the PISP nursery on vegetative propagation of Acacias.

A first experiment focused on the rooting ability of non-juvenile material of Acacia mangium x auriculiformis hybrids. Height 1.5-year old trees in Luasong were coppiced and cuttings introduced in the mist for rooting. Average rooting rate was 45%, while the global success of introduced cuttings was 35% (accounting for mortality during weaning). The results are presented in Appendix 11.

A second experiment on coppicing and vegetative propagation ability of young stockplants of *A. crassicarpa* and *A. mangium* was carried out. It was found that cuttings of both *A. crassicarpa*

and *A. mangium* roots and survive well, with a global success rate of 64 and 40% respectively (Appendix 12).

2.1.6. Vegetative propagation – Introduction of *in vitro* material

Global details of the Acacia materials transferred from the PBL to PISP, acclimatised and transplanted, with rooting and survival rates per species are given in Appendix 13.

2.2. OCTOMELES SUMATRANA (BINUANG)

The two silviculture trials (weeding and fertilizing) planted in 1995 at Taliwas were concluded in 1996. The trials were then converted to a single thinning trial in July 1998.

The boundaries separating the two previous silviculture trials were removed and new blocking was done taking into consideration the growth variation across the area. The whole area was currently subdivided into 5 homogenous blocks and three thinning regimes were used, i.e. 20%, 30% and 40% of the number of trees. The tree basal areas were estimated before and after the thinning operation. The report and maps of the new treatments are attached in Appendix 14.

2.3. TECTONA GRANDIS

2.3.1. Field trials – Comparison of propagation methods

A first trial to compare different propagation methods of teak was established at KM18, Taliwas in September 1998. The three propagation methods were by seeds, cuttings and *in-vitro* micro propagation. The map of the trial is attached in Appendix 15.

A second, more scientifically accurate trial is under preparation, based on the use for the three propagation methods (cuttings, in vitro and seeds) of the same homogeneous material (seeds). Fresh seeds were purchased from FRC (provenance Kota Marudu) and half of them germinated both in Luasong and in Taliwas. Once the seedlings are tall enough, conventional and micro cuttings will be collected from them and introduced respectively under the mist system and in *in vitro* conditions in the PBL. In this phase, each genotype should be identified and propagated with the same care along the cutting / tissue culture operations.

Once the cuttings and the tissue cultures from this material are well established, the second batch of the same seeds (at present stored in the fridge) will be germinated, and new seedlings raised. Such procedure should allow comparing in the field seedlings, micro- and macro-cuttings of the same genetically homogenous materials.

To date, 154 seedlings are available and ready to be propagated by the PBL. A total of 100 different genotypes should be individually propagated to enable a robust statistical comparison

2.3.2. Commercial Production

The commercial production of teak was re-activated in Luasong following to demand from buyers. The PISP was again involved in the production of teak cuttings and acclimatisation of plantlets from the Plant Biotechnology Laboratory. The production target from PISP is about 4.000 saleable Solomon's teak per month.

The operation started in February 1999 and after one and a half month, about 12.000 cuttings were made mainly from the hedge gardens in the Demo Plot. The current rooting success is about 60%.

2.3.3. Assessments - Provenance/ progeny trials

Two provenance/progeny trials were established with teak in 1997, one in Taliwas and one in Luasong. Both of the trials have been measured in June 1998, and the Taliwas trial has also been thinned in October 1998. Following some sanitary problems (wind breakages, pest attacks,

bendings, etc.), the Taliwas trial has been assessed again in April 1999. The results of this last assessment are reported in Appendix 16. The main results are that the survival rate was 89%, 18% of the trees had one or more major trunk defects, 37% only one or two minor defects and the remaining 45% were completely healthy (excluding possible stem borer holes that were not recorded on this study; the study of the shoot borer in this trial is being conducted by Kotulai, Pest and Disease Unit, ICSB).

Flowering

In October 1998, the flowering patterns of the two provenance/progeny trials of teak in Luasong and Taliwas have been assessed. Only a small portion of the trees is flowering, about 1% in Luasong and 2.6% in Taliwas.

2.3.4. Silviculture

The silviculture of Teak, with a special regard to its growth in Sabah as compared to other world's regions, was studied and the results published in a paper entitled "Growth Performance of Teak". The study required two visits to the Forest Research Centre (Sepilok) for discussion with the personnel in charge of the silviculture plots established in Sabah by the Forestry Department and collection of data. The data from Sabah were statistically treated in Luasong, and compared to other data available in the literature for Ivory Coast, Latin America, India and other world regions.

2.3.5. Thinning of the Demo Plot

The 4-year-old stand of *in-vitro* germinated Perlis' Teak at the demo plot was thinned in June, reducing the initial number of trees (299) of about 50%.

2.3.6. Collection of new plant materials

A mission to Mata Ayer, Perlis was organised by the PBL in May 1998 with the objective to collect tissues from adult selected teak trees. M. Schueller from PISP participated to this mission. Plant materials were collected from twenty-two trees and introduced in *in vitro* at the PBL for further propagation.

2.4. KHAYA IVORENSIS

There are three plots being maintained for this species. A provenance trial was established in open planting, and the two progeny trials in line planting.

2.4.1. Provenance trial

The provenance trial (KIV1) includes three provenances from Ivory Coast (Bonuoa, Mopri and Yapo) and one provenance from Kulim, Kedah and was established in September 1990. The design was a Randomized Complete Block with 3 x 3 trees per plot and 10 replications on the same location. Thinning was conducted in December 1996 to reduce the plot size to 5 trees.

The growth performances of the Ivory Coast provenances are better than the Kulim provenance (Table 5). This variation is consistent since the early age of the stand.

Table 5: Growth of 8.3 years old *Khaya ivorensis* – Open planting (KIV1)

Provenance	Mean Height (m)	Mean DBH (cm)	HMAI (m/yr)	DMAI (cm/yr)
Bonuoa	18.4 (2.2)	21.0 (4.2)	2.22	2.53
Mopri	18.8 (1.5)	22.3 (3.1)	2.27	2.69
Yapo	18.8 (1.6)	21.9 (3.1)	2.27	2.64
Kulim	17.0 (2.0)	17.6 (3.6)	2.05	2.12

Note: Value in parenthesis indicates standard deviation

Some of the trees (8 out of 190) in the provenance trial plot were seen fruiting in January 1999. Germination test indicates 73% seed viability, which was considered good for first fruiting. At present in the Luasong nursery there are about 900 seedlings collected on 8 fruiting trees. A pest attack was recorded on this trial in 1998 by Kotulai (Pest & Disease Unit, ICSB).

2.4.2. Progeny Trials

The progeny trials of *K. ivorensis* were established in 1991 using seedlots from Ivory Coast (Bonoua, Mopri and Yapo provenances). In the line planting under logged-over forest, the distance between the planting points was 4.5 m and 9 m apart between the rows. The first trial (KIV3) tested 9 progenies in a 3 x 3 Balanced Lattice design and the second (KIV4) tested 12 progenies in a Rectangular Lattice design. All the 9 progenies in KIV3 were included in KIV4.

As in the previous years, KIV3 performed better than KIV4 (Table 6 and 7). Both trials were established near to each other but the forest shade for KIV4 is heavier than in KIV3 due to the presence of larger trees.

The performance of *K. ivorensis* in the open planting was undoubtedly better than the growth in line planting. The variation in growth performance in KIV3 and KIV4 further suggests that this species is sensitive to heavy shade by forest trees.

Table 6: Growth of 7.7 years old *K. ivorensis* – Line planting (KIV3))

	No. of Living Trees	Survival (%)	Mean HT (m)	Mean DBH (cm)	HMAI (m/year)	DMAI (cm/year)
Average	302	84	12.9 (3.7)	11.3 (4.6)	1.67	1.47

Note: Value in parenthesis indicates standard deviation

Table 7: Growth of 7.7 years old *K. ivorensis* – Line planting (KIV4))

	No. of Living Trees	Survival (%)	Mean HT (m)	Mean DBH (cm)	HMAI (m/year)	DMAI (cm/year)
Average	147	82	7.5 (3.9)	6.8 (3.8)	0.97	0.88

Note: Value in parenthesis indicates standard deviation

2.5. XYLIA XYLOCARPA

Xylia xylocarpa is a heavy hardwood that occurs naturally in India, Myanmar, Indo-China and Thailand. Its hard and durable wood is normally used for heavy construction purposes. This species was planted in September 1990 as an introduction species both in the line and open planting in Luasong. It gained recognition as a potential plantation species due to its fast growth and good stem form especially in the open planting plot. The species performed even better than *Khaya ivorensis* in the open planting, although with greater wood density (*X. xylocarpa* > 800 kg/m^3, *K. ivorensis* >< 450 kg/m^3).

As for *K. ivorensis*, the growth of *X. xylocarpa* on open planting was far better than in line planting under logged-over forest (Table 8).

Table 8: Growth of 8.3 years old *Xylia xylocarpa*

Trial	No. of Living Trees	Mean height (m)	Mean diameter (cm)	HMAI (m/yr)	DMAI (cm/yr)
XXY1 (Open-planting)	37	22.7 (2.2)	21.2 (3.0)	2.74	2.55
XXY2 (Line-planting)	101	9.7 (3.3)	9.3 (4.1)	1.17	1.12

Note: Value in parenthesis indicates standard deviation

2.5.1. Vegetative propagation - *Khaya ivorensis* and *Xylia xylocarpa*

Vegetative propagation experiments were done on *Khaya ivorensis* and *Xylia xylocarpa*. Detailed reports are presented in Appendix 17, 18 ans 19. The findings for 1998 are as follows:

1. *K. ivorensis* and *X. xylocarpa* could be mass propagated by cuttings.
2. Rooting success of cuttings from bulk of seedlings of *K. ivorensis* was high, i.e. more than 80%.
3. Rooting success of cuttings from coppices of 7-year-old *X. xylocarpa* averaged 47%.
4. Cuttings of both species needed between six to twelve weeks to root on the misting beds.
5. There was an effect of cutting order in *X. xylocarpa* but negligible for *K. ivorensis*.
6. For both species, there were significant differences in rooting among clones

Experiments of inducing coppices on older trees (8 years old) without felling the trees are on going for both of the species.

3. OTHER MATTERS

3.1. Renovation of misting systems

There are two misting systems under the management of PISP. Both misting units were renovated to suit the sanitation and good functioning requirements for vegetative propagation purposes. The smaller unit (Misting No. 1) which is located near the office building has two misting beds and one weaning bed. The larger Misting No. 2 and 3 are at the research nursery, consist of eight misting beds and two weaning beds. All the misting facilities enable PISP to accommodate about 10,000 cuttings or plantlets at any particular time.

3.2. Sampling for wood properties studies

The Wood Section Unit of the Forest Research Centre (FRC) at Sepilok proposed to conduct wood property investigations on *Xylia xylocarpa* in Luasong. An agreement was made, and *Khaya ivorensis* was also included in the studies as both species are important to ICSB.

Five trees of each species were felled in November 1999 and wood samples brought to the FRC for mechanical and chemical properties investigations. Detailed reports are yet to be received from FRC.

3.3. International Projects: TS3*-CT94-0285 "Conservation, Genetic Improvement and Silviculture of Rattan Species in South East Asia" (EEC-STD3)

The European funded project on rattans, which reunited from 1994 to 1998 the research sector of several private (ICSB) and government (CIRAD-Forêt, FRIM, FRC, Royal Botanic Gardens, Kew) organisations, come to its end in June 1998. All participants contributed a final scientific and financial report, and the coordinator (CIRAD-Foret) edited a final scientific report to be submitted

to the EU. In November 1998, the EU communicated that the work was considered successfully conducted and concluded.

In order to bring to the attention of a wider public the results of the project, and also to discuss the complicate economy of the rattan industry (from government policy to planting to marketing), a Consultation of Experts was organised in Kuala Lumpur with the help of FRIM, ICSB and Yayasan Sabah.

3.4. Meeting Organisation

The PISP, together with FRIM, organised an "International Consultation on Rattan Cultivation: Achievements, Problems and Prospects" from the 12 to 14 of May 1998 in Kuala Lumpur. The meeting, financed mainly by the European Community, CIRAD-Foret and FRIM, brought together around 60 experts from 8 Asian and European countries to discuss economic and biological problems of rattan cultivation.

Twenty-four communications were presented at the meeting from resource persons including policy-makers, researchers, industrial planters and state foresters. All the communications have been collected in the written form, submitted to international peer review, and will be soon published into the Proceedings of the meeting.

3.5. Missions

A foreign mission for technical support in aerial photography interpretation was organised by PISP, financed by the Ministry of Foreign Affairs of France and carried out by a CIRAD-Foret's expert, Mrs. Pain-Orcet from 17 to 27 of April, 1998. The mission visited the Cartography Unit (Kota Kinabalu) and the Plantation Unit (Luasong) of ICSB; the first four days were dedicated to meeting the direction and personnel of ICSB, to the study of maps, aerial photographs and the equipment of ICSB. The following two days were spent in the field to check the quality of the photographs and the interpretation previously done in the office. The mission gave origin to a mission report in English.

M. Schueller participated to a mission to Perlis (Peninsular Malaysia) organised by the PBL with the objective to collect tissues from adult selected teak trees. The material has been introduced in *in vitro* at the PBL for further propagation.

Three other meetings were attended:

International Workshop on Forestry and Climate Change: Cost Effective Credible Emission Reduction Opportunities. October 22-24, 1998, Luasong Forestry Centre, Malaysia.

Seminar on High-Value Timber Species for Plantation Establishment - Teak and Mahoganies. December 1-2, 1998, Tawau, Malaysia.

Annual Scientific Week of CIRAD-Foret, September 1-5, 1998, Montpellier, France

3.6. Invitation of a rattan expert to Tawau

In 1998 CIRAD-Foret and ICSB invited at the Luasong Forestry Centre a rattan expert, Mr. D. Hauri, to present his work on rattan economy and market in Indonesia. Mr. Hauri is a GTZ researcher (Germany) that worked for several years at the "Promotion of Sustainable Forest Management Systems" Project in East Kalimantan, under the Indonesian-German Technical Cooperation. He presented his interesting findings on how the Indonesia Government policy of restrictions on rattan market influences the rattan price over the South East Asian region.

3.6. Publications

Pouet K.. 1998. Characterisation of the light and competition environment in a line planting of *Calamus subinermis* under logged-over forest in Sabah. Ecole Nationale d'Ingenieurs des Travaux Agricoles de Bordeaux, France.

Bacilieri R.. Alloysius D., Lapongan J., *in press.* Growth performance of Teak. Paper presented at the "Seminar on High-Value Timber Species for Plantation Establishment – Teak and Mahoganies" 1-2 December, 1998, Tawau, Malaysia.

Bacilieri R., Maginjin B., Pajon P. and D. Alloysius, *in press.* Silviculture of Rattans under Logged-over Forest. Paper presented at the "International Consultation on Rattan Cultivation: Achievemnts, Problems and Prospects". 12-14 May 1998, Kuala Lumpur, CIRAD-Foret / FRIM.

Pain-Orcet. M. 1998. Aerial Photo Interpretation - Mission Report to Sabah. Malaysia. CIRAD-Foret, France

Bacilieri R. 1998. Final Scientific Report of the Project: Conservation. Genetic Improvement and Silviculture of Rattan Species in South East Asia. Commission of the European Community, CIRAD-Foret.

Bacilieri R., Appanah S., *in preparation.* Proceedings of the"International Consultation on Rattan Cultivation: Achievements, Problems and Prospects". 12-14 May 1998, Kuala Lumpur, CIRAD-Foret/FRIM.

Bacilieri R., Galiana A., Monteuuis O., Goh D.. 1999. Propagation of selected Teak plant material in Sabah - A collaborative effort between ICSB and CIRAD-Foret. Poster presented at the FAO-FORSPA / Teaknet Seminar: Site, Technology and Productivity of Teak Plantations. January 26-29, 1999, Chiang Mai, Thailand.

Appendix 1

Trials/seedstands of *C. manan* at Luasong Forestry Centre (as end of March. 1999)

Trial/ seedstand no.	Date of planting	No. of provenance/ progeny	Total plants	Planted area (ha)
Progeny 1 (CMB1)	Feb 92	5	100	0.20
Progeny 2 (CMB2)	Feb 92	4	104	0.20
Progeny 3 (CMB3)	Feb 92	3	90	0.10
Progeny 4 (CMB4)	Jan 93	20	1000	1.25
Progeny 5 (CMB5)	Jun 94	20	400	0.50
Progeny 6 (CMB6)	Jun 94	6	180	0.22
Progeny 7 (CMB7)	Jun 94	9	180	0.22
Progeny 8 (CMB8)	Jun 94	30	300	0.38
Progeny 9 (CMB9)	Nov 94	7	210	0.26
Resource 1	Jun 94	6 (bulk)	197	0.25
Resource 2	Jun 94	14	256	0.32
Resource 3	Jun 94	8	244	0.31
Resource 4	Jun 94	12	310	0.39
Resource 5	Jun 94	17	170	0.21
Resource 6	Nov 94	6	300	0.38
Progeny 10 (CMB10)	Aug 95	36	288	0.36
Resource 7	Dec 97	2	120	0.15

Appendix 2

Trials/seedstand of *C. subinermis* at LFC (as end of March, 1999)

Trial/ seedstand no.	Date of planting	No. of provenance/ progeny	Total plants	Planted area (ha)
Progeny 1 (CSB1)	Jul 91	5	75	0.10
Progeny 2 (CSB2)	Jul 91	3	165	0.20
Progeny 3 (CSB3)	Jul 91	6	240	0.30
Progeny 4 (CSB4)	Dec 91	5	150	0.19
Progeny 5 (CSB5)	Feb 93	14	700	0.90
Progeny 6 (CSB6)	Jun 94	6	180	0.22
Progeny 7 (CSB7)	Jun 94	30	450	0.56
Progeny 8A (CSB8A)	Nov 94	72	432	0.54
Progeny 8B (CSB8B)	Nov 94	72	432	0.54
Provenance 1 (CSC1)	Dec 90	6	420	0.63
Resource 1	Jun 94	4 (bulk)	200	0.25
Resource 2	Jun 94	9	170	0.21
Resource 4	Dec 94	74	394	0.49
Progeny 9 (CSB9)	Aug 95	20	200	0.25
Resource 3	Aug 95	1	50	0.06
Progeny 10 (CSB10)	Oct 96	16	448	0.56
Resource 5	Oct 96	6	120	0.18
Resource 6	Dec 97	11	227	0.28
Resource 7	Jul 98	14	321	0.48

Appendix 3

Trials/seedstand of *C. caesius* at LFC (as end of March, 1999)

Trial/ seedstand no.	Date of planting	No. of provenance/ progeny	Total plants	Planted area (ha)
Progeny 1 (CCB1)	May 91	43	645	1.00
Progeny 2 (CCB2)	May 91	35	525	0.80
Progeny 3 (CCB3)	Jun 91	25	625	0.90
Progeny 4 (CCB4)	Sep 91	10	400	0.60
Progeny 5 (CCB5)	Dec 91	40	600	0.90
Progeny 6 (CCB6)	Dec 91	35	700	1.10
Progeny 7 (CCB7)	Dec 91	33	660	1.00
Provenance 1 (CCC1)	May 92	9	270	0.30
Resource 1	Jun 91	60	300	0.45
Resource 2	Sep 91	10	50	0.08
Resource 3	Dec 91	40	200	0.30
Resource 4	Dec 90	1 (bulk)	100	0.15
Resource 5	Jun 94	1 (bulk)	50	0.01
Resource 6	Aug 95	1 (bulk)	50	0.06
Resource 7	Aug 95	1	100	0.13
Progeny 8 (CCB8)	Oct 96	16	400	0.60
Progeny 9 (CCB9)	Oct 96	16	432	0.67
Resource 8	Oct 96	2	40	0.06

Appendix 4

Trials/seedstand of *C. ornatus* at LFC (as end of March, 1999)

Trial/ seedstand no.	Date of planting	No. of provenance/ progeny	Total plants	Planted area (ha)
Progeny 1 (COB1)	Aug 95	20	180	0.23
Resource 1	Aug 95	1	100	0.06
Progeny 2 (COB2)	Dec 97	30	270	0.34
Resource 2	Jul 98	3	90	0.14

Trials/seedstand of *C. optimus* at LFC (as end of March, 1999)

Trial/ seedstand no.	Date of planting	No. of provenance/ progeny	Total plants	Planted area (ha)
Resource 1	Aug 95	2	187	0.28
Resource 2	Dec 97	5	150	0.19

Appendix 5

Calamus subinermis yield plots. Estimation of the length of mature cane.

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Introduction

In 1991, 13 yield plots for *Calamus subinermis* were planted in LFC. They were assessed at year intervals in order to follow the growth of the plants. In this paper, the results of the last assessment, made in June 1998, and an estimation of the expected income in 1998 are presented and compared the data to the previous assessments,.

Material and method.

The yield plots were planted with the line planting method, with 2 rows of 15 plants each in a stripe open in the forest by felling all the non-commercial species, lianas and bamboos. The original density was of 800 plants/ha. The plots were assessed regularly every year since planting, with the exception of 1997 when the assessment could not be carried out. The last assessment occurred in June 1998. When the stems were too long to be measured with the conventional tape, we used the method of Stockdale & Power (1994; see the paper on *C. caesius* yield plots, this volume).

We first present the results of the last assessment, then the evolution of the mean length from 1992 to 1998. A growth model was computed assuming that the growth follows a sigmoid pattern (Nasi, 1990). Finally, an estimation of the length of mature cane was computed, based on the maturation data recorded for *C. caesius* in a 6-year old plot in LFC.

Results and analysis

Results of the last assessment of June 1998.

Table 1 gives a summary of the data recorded on the 13 yield plots and is illustrated by Figure 1 that shows the average length per plot compared to the maximal length recorded on the same plot. Most of the time the maximal length is far above the mean. However the data showed less heterogeneity than for *C. caesius* (See *C. caesius* yield plots). Figure 2 shows the distribution of the plants all over the plots.

Table 1 : Average, maximum and minimum length of the main stem per plot (in cm), and survival recorded in the yield plots.

Plot	Avg lgth	Max lgth	Min lgth	Nb dead	% dead
1	1340	6307	28	12	40%
2	157	470	15	9	30%
3	1452	4050	18	6	20%
4	73	370	20	14	47%
5	553	1820	20	9	30%
6	471	2590	10	7	23%
7	793	2907	15	11	37%
8	44	95	15	2	7%
9	30	85	10	3	10%
10	208	1070	40	5	17%
11	33	65	10	2	7%
12	94	455	5	3	10%
13	23	63	5	10	33%

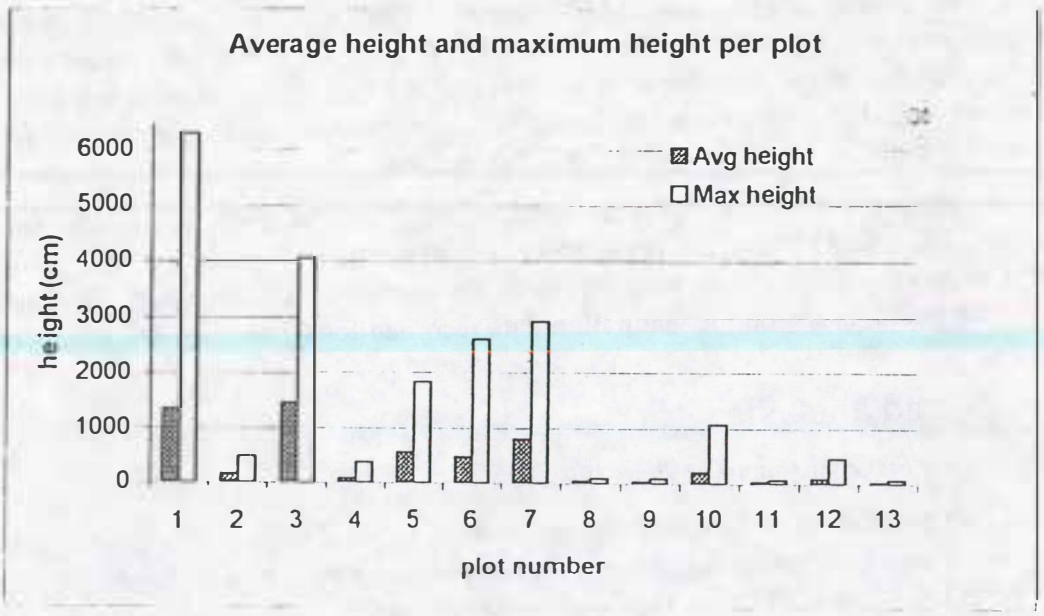

Figure 1 : Average and maximum length per plot.

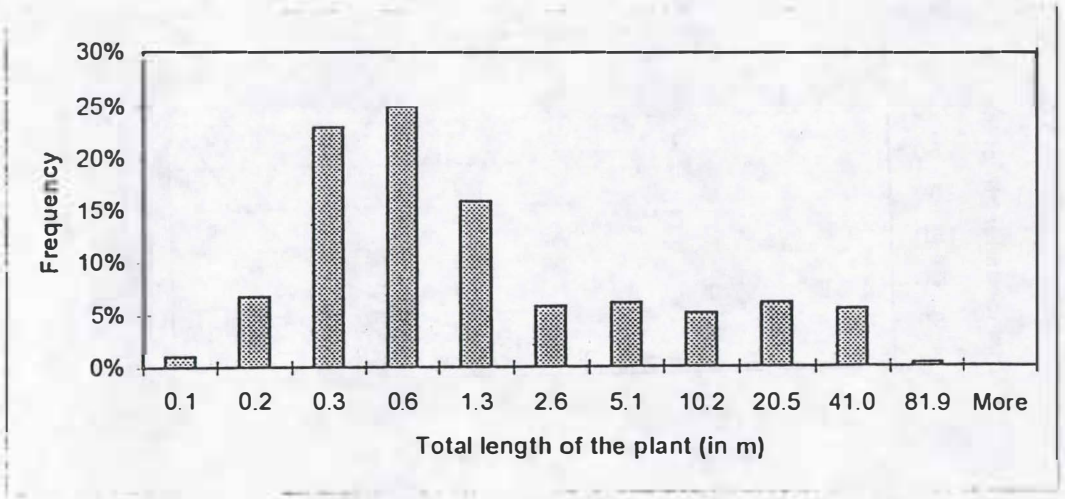

Figure 2 : Distribution of the plants by classes for the 13 plots.

Comparison of the growth to the previous assessments

Table 2 gives the results of the previous assessment compared with the last one in 1998. The average length of the plants has increased following an exponential trend, and the standard deviation among plots has also increased of a comparable quantity. Figure 3 shows the growth of the total mean length of the plants in the plots and the curve trend of the growth.

Table 2 : Mean length of the plants per plot and per assessment (in cm).

Plot	1992	1993	1994	1995	1996	1998
1	9	26	52	106	156	1340
2	4	7	10	14	16	157
3	9	34	127	269	411	1452
4	5	10	15	15	18	73
5	7	14	27	56	91	553
6	6	12	27	56	84	471
7	6	14	31	73	119	793
8	4	8	10	12	13	44
9	3	4	7	8	9	30
10	5	11	17	28	41	208
11	2	4	6	7	9	33
12	4	7	12	19	26	94
13	2	3	3	3	4	23
Average	5	12	26	51	77	377
Stdev	2	9	33	72	112	501

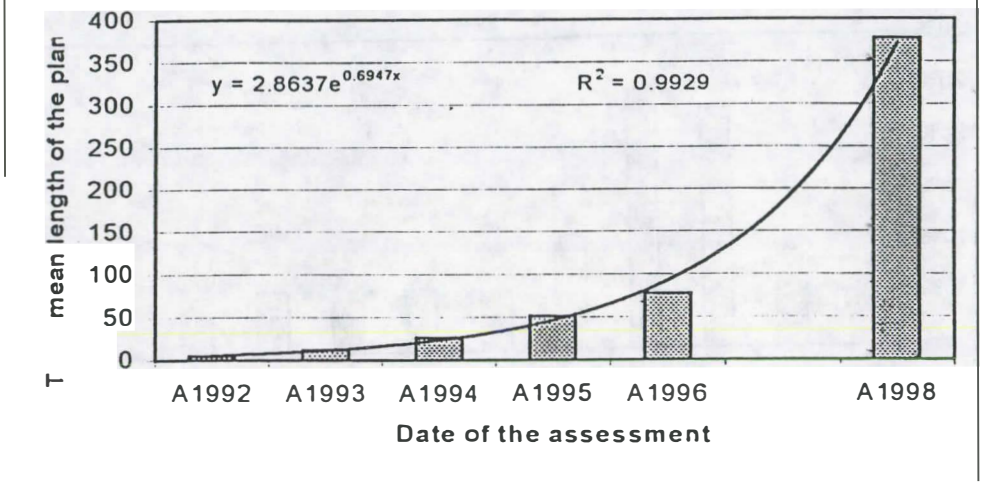

Figure 3 : Evolution of the growth (in cm) from 1992 to 1998.

Estimation of the length of mature cane and the expected income in 1998.

The estimation of the length of mature cane was made using previous data on cane maturation collected by Nasi (1992) on *C. manan*. We then calculated the number of 4-meter long mature sticks that may be obtained from the total length of mature cane (Table 3). The mean standard price of 4-meter long pieces was estimated to be 2 RM (price in Tawau in 1997); this is a conservative estimation as the rattan prices in Tawau are among the lowest in Sabah; for example, in Kota Kinabalu the price for *C. subinermis* was twice the price in Tawau in 1997. Our revenue estimations do not include the neither planting, maintenance costs, nor the harvesting cost (Table 3).

Discussion

The heterogeneity between plants even in the same plot is a common feature of the rattan plantation, often observed in Luasong Forestry's plantations. Along the rows, one plant may reach more than 30 meters while the next one in the row may still be in the rosette stage Since the size of the *C. subinermis* yield plots is very small, it may be the proof that even in homogeneous plot genetic and individual physiological characteristics play a key role in the growth of rattan, besides the environmental factors.

The growth pattern up to 7 years shows an exponential trend. Assuming that the global growth follows a sigmoidal pattern, with a first initial stage of slow growth after the rosette stage, then a period of intense growth before the final slowdown, the data recorded in the yield plots up to 1998 showed that we are still in the first stage of the growth, and that the period of intense linear growth has not yet occurred. We may expect it to occur soon, according to the model computed. Over the first 7 years, the heterogeneity between the plants has also increased, the coefficient of variation ranging from 40% in 1992 to 133% in 1998.

Table 3 : Estimation of the length of mature cane and expected revenue.

Plot	AvgLgth	TotLg	TotMat	TotLg/ha	TotMat/ha	Nb of sticks/ha	Revenue/ ha
1	13.40	241.13	108.51	6430	2894	723	1447
2	1.57	32.89	14.80	877	395	99	197
3	14.52	348.38	156.77	9290	4181	1045	2090
4	0.73	11.75	5.29	313	141	35	71
5	5.53	116.22	52.30	3099	1395	349	697
6	4.71	108.32	48.74	2888	1300	325	650
7	7.93	150.76	67.84	4020	1809	452	905
8	0.44	12.25	5.51	327	147	37	74
9	0.30	8.15	3.67	217	98	24	49
10	2.08	52.02	23.41	1387	624	156	312
11	0.33	9.10	4.10	243	109	27	55
12	0.94	25.38	11.42	677	305	76	152
13	0.23	4.68	2.11	125	56	14	28
Avg	4.05	92.63	38.80	2470	1035	259	517

Legend: **AvgLgth(m)**=Average stem length of the plot; **TotLg(m)**= Total length per plot; **TotMat(m)**= Total length of mature cane per plot, estimated from data for *C. caesius*; **TotLg/ha(m)**=Total length calculated for 1 ha; **TotMat/ha(m)**=Estimated total length of mature cane per ha; **Nb of sticks/ha**=Estimated Nb of 4 m sticks of mature cane to sold per ha; **Revenue/ha(RM)**=Total income from harvesting per ha (1 stick=2RM; production costs not subtracted).

From the yield plot data and assuming a percentage of mature cane of 45% (Nasi 1992), we computed an estimation of the percentage of mature cane and an expected income of around 500 RM/ha, from which must be subtracted all the planting, maintenance and harvesting costs.

Conclusion

Seven years after establishment, the *Calamus subinermis* planting at Luasong is still not ready to harvest. However, the plants are undergoing a steady increase of the growth rate, and it is possible that in the near future the revenue may be positive. Apparently the prospect for *C. subinermis* is more attractive than that for *C. caesius*.

Further improvement can be obtained by better adapting site-matching, maintenance and silviculture techniques to the Luasong conditions. Still, further information from harvesting in real plantation conditions are required to confirm the viability of the planting.

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Absence of detectable genetic effects in the *Acacia mangium* x *auricoliformis* hybrid clonal trial (Ah18c) in Brumas

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Introduction

Following new contacts established between the Plant Improvement and Seed Production Joint Project of CIRAD-Foret and Innoprise Corporation Sdn. Bhd. (PISP), and the Research Section of Sabah Softwood Sdn. Bhd. (SSSB), a scientific study has been carried out on the genetic differences among clones of *Acacia mangium x A. auriculiformis* hybrids.

In several occasions in forest trees cultivation, hybridisation has meant increased wood production, especially for short rotation wood species such as *Eucalyptus* and *Populus*. Several independent genetic mechanisms may play a role in conferring better performances to the hybrid, the most common consisting in hybrids accumulating positive and complementary qualities (genes) of the two parent species. For example, in diploid species as trees, if species 1 carries genes *AA* and *bb*, and species 2 carries *aa* and *BB*, the hybrid will become *Aa* and *Bb*, *A* and *B* being both favourable and dominant over *a* and *b*).

In a hybrid population, a certain amount of variation can still be found, because the quality of a particular tree depends upon the random association of both positive and negative parental genes. In addition, second generation progenies originated by hybrid matings are extremely variable because of random segregation at recombination (for example, from a cross between two hybrids *AaBb*, an offspring tree can inheritate all "small letters" genes, becoming *aa* and *bb*, *a* and *b* standing here for recessive and deleterious genes). For these reasons, clonal propagation of the best hybrids is a preferential way to preserve the genetic advantage of selected hybrid trees.

Based on this theoretical background and on the previous success of other hybrid species, Acacia hybrids have for long time drawn the attention of researchers. However, few results have been published so far, and it is not clear if this is due to the secrecy built by industrial companies around new advances in genetic improvement of Acacias, or to the fact that Acacias belong rather to those species that do not display hybrid vigour.

A clonal trial of *Acacia mangium x A. auriculiformis* hybrids has been established in 1993 in Brumas by SSSB. Unfortunately, the trial does not include a control with the parental species, so that it is not possible to directly assess the advantage of hybrids upon their parent trees. Nevertheless, the study of the trial can cast more light on the performances and the variability of Acacia hybrids. An indirect comparison with the two parental species can be obtained by using the data available in SSSB on the performances of pure *A. mangium* and *A. crassicarpa* stands planted in similar soils at the same planting date.

Material and Methods

The trial Ah18c planted in Brumas over an area of 0.84 ha includes 144 clones produced from seedlings collected in 5 clonal bi-specific (*Acacia mangium* and *A. auriculiformis*) seed orchards. It is worth noting that the clonal bi-specific orchards all contained a very small number of clones (genotypes) per species, as it is visible from the dominance of just two trees (AA7d and AA7f) among the maternal parents of the hybrid clones (Table 1).

In 1992, the seeds collected in these orchards were germinated, and a number of putative hybrid seedlings were selected based on their intermediate morphology. One hundred forty four of these seedlings were multiplied by vegetative propagation, and the cuttings were established in the field in September 1993. Each individual tree was identified by its own clone number and by a code indicating the maternal plant from which it originated. The code of the maternal trees includes a reference to the species, AA standing for *Acacia auriculiformis* and AM for *Acacia mangium.*

The experimental design was a single-tree randomised complete block design with 6 repetitions. Being a single-tree design, each repetition included only one copy of a given clone, so that over all six repetitions there were only 6 trees per clone. The trial has been assessed every year since planting. In this paper we have only studied the assessment of 1998; the other assessments were useful to trace the history of the trees and correct mistakes in data recording. For example, trees recorded as "slashed" in 1994 and as "alive" in 1998 were deleted from the analysis, being young re-sproutings.

The measured characters were :

- Diameter at the breast height
- Height
- Straightness (score from 1=straight to 6=extremely crooked)
- Fork (scores: 1=no fork; 2=one fork in the upper portion of the trunk; 3=one fork in the medium portion of the trunk; 4=one fork in the lower portion of the trunk)
- Number of forks (scores: 1=no fork; 2=1fork; 3=2forks; 4=3forks)
- Branch persistence (scores from 1=self-prunning tree to 6=very persisting branches)
- Branch size (scores from 1=very small branches to 6=very large branches)
- Branch angle (scores from 1=horizontal to 5=parallel to the tree; this is a special interest because the more a branch is vertical, the higher the probability for it to become a leader)

In addition to these characters, we also calculated the volume of the trees according to the cone formula. This formula is considered very imperfect, both because it does not take in account the true form of the trunk, and because being based on the total height of the tree it makes no reference to the length of the usable bole. Volume tables for Acacia hybrids do not exist yet, and the present formula, in spite of its defects, is the only known way to jointly take in account the effect of both diameter and height growth in ranking the trees. A better formula can be used in the future based on true trunk measurements.

The model chosen for the analysis of variance was as follows:

single tree measurement = repetition effect + maternal tree effect + clone within maternal tree effect + error

Table 1. Average values for the progenies of each maternal parent represented in the trial.

maternal tree	number of planted trees	percent survival	diameter	height	straight.	fork	number of forks	branch persistance	branch size	branch angle	volume (m^3)
AM7c	54	26%	17.2	16.0	3.2	1.8	1.6	3.3	3.0	3.2	0.19
AM7b	24	29%	16.1	16.6	3.4	2.3	1.7	3.1	3.1	3.5	0.17
AA7d	168	34%	16.4	15.7	3.2	2.0	1.6	3.2	2.9	3.2	0.16
AA7f	570	29%	16.0	15.5	3.4	1.9	1.6	3.1	2.8	3.0	0.16
AA7h	48	42%	15.2	15.5	3.3	1.8	1.4	3.0	2.9	3.1	0.14

In a single-tree plot, the analysis of the interaction between repetition and clone or maternal tree is not possible due to the absence of within-experimental unit variation.

Results

The trial has been affected by a large amount of mortality (57%), which reduced further the (already small) number of trees per treatment. Due to this, the statistical analysis of the 1998 assessment did not yielded significant differences among clones or maternal parents for any of the studied characters (an example of the results of the analysis of variance is given in Table 2).

Table 2. Analysis of variance for the height. The other characters gave analogous results of absence of significant effects in the trial (not shown).

Source	DF	Sum of Squares	F Value	Prob. of error
Model	80	971.06	0.98	53 %
Repetition	5	69.54	1.12	35 %
Maternal tree	4	12.12	0.24	91 %
Clone	71	873.49	0.99	50 %
Error	184	2281.19		

Statistical tests being not significant, selection can either focus on the trees that survived well (>83%: for example clones n. 41, 68, 8, 60, 131, 78), or on those with an acceptable survival (>50%) and with the maximum volume (clone n. 116, 72, 37, 46, 51, 79). Overall, the best clone seem to be n. 41, collected on an *A. auriculiformis* maternal parent tree [AA7d]; in the trial, clone n. 41 has 100% survival and is only eighth (out of 144) in the ranking for volume (Table 3). The ranking of the trees according to their maternal parent did not add much information, the best parent tree in terms of volume being the worst in terms of survival and *vice versa* (Table 1).

Some phenotypic correlations were observed among characters (Table 4), the most interesting being that the largest trees have the larger number of forks, and the taller trees being also the more straight. Genotypic correlations could not have been calculated because of the lack of significance of the statistical tests of difference among clones.

Table 4. Phenotypic correlation coefficients among characters.

	Diamet.	Height	Straight.	Fork	Number of forks	Branch persistence	Branch size	Branch angle
Diameter	1							
Height	0.779	1						
Straight.	-0.204	-0.282	1					
Fork	0.362	0.192	0.181	1				
Number of forks	0.408	0.193	0.249	0.868	1			
Branch persistence	0.079	-0.025	0.153	0.047	0.109	1		
Branch size	0.076	0.051	0.090	-0.009	0.046	0.012	1	
Branch angle	-0.013	0.048	-0.081	-0.072	-0.030	-0.049	0.354	1

It is worth at this point to study what is the growth of the best hybrid clones 4 years and ten months after establishment. To do so, one can for example take the survival and growth of the ten best hybrid clones (36 trees); in this case, the average plot has a survival rate of 60%, and the average tree a diameter, height and volume (based on the cone formula) of 20.7 cm, 18.2 m and 0.31 m^3 respectively. These average values can be compared to the average measurements of the 40 best trees per hectare in pure *A. mangium* or *A. auriculiformis* stands of the same age and established on approximately the same soil in Brumas.

Conclusion

The most remarkable point of this analysis is that the trial failed to capture any existing difference among clones. This does not mean that there are no differences, just that the trial was not powerful enough for the discrimination. The main reason of the lack of power of the experimental design was the small number (6) of tree per treatment. Additional weakness was added by the high level of mortality (57%) and by the lack of control with the two pure parental species.

Due to these problems, it was impossible both to establish a robust selection of the best hybrids, and to confirm or reject the hypothesis of the superiority of hybrids as compared to true species. Our observations indicated that many trees presented important trunk defects such as non-circularity of the trunk section, pest and disease attacks and dieback that brought the affected tree to death. One reason behind such pattern may be the presence within the hybrids of selfings. Selfings may originate in bi-specific seed orchard when one tree is self- rather than cross-pollinated. Asynchrony of flowering between the two species may induce a flowering tree to self-pollination, especially if it has many copies of itself around it, as it is the case when clonal bi-specific orchards are established with few clones only.

For selection purposes, it was nevertheless possible to discriminate few clones showing both a good survival and growth. These clones should now be multiplied again (with the help of *in vitro* techniques for example) and tested against ordinary material such non-selected hybrids and pure species. In the field, the problem of high mortality in Acacia stands (a similar problem was found in an *Acacia crassicarpa* trial) remains to be addressed.

Table 3. Average values for the clones in the trial. Only clones with more than 50% survival are represented.

clone	maternal tree	number of living trees	diameter	height	straight.	fork	number of forks	branch persistance	branch size	branch angle	volume (m^3)
116	AA7f	3	23.0	18.9	3.3	3.0	2.7	2.8	2.7	3.3	0.39
72	AA7f	4	21.4	19.1	3.0	2.4	1.7	2.7	2.5	2.7	0.34
37	AA7d	3	20.8	19.0	3.0	2.3	1.6	3.6	2.6	2.3	0.32
46	AA7f	3	20.4	18.9	3.0	1.0	1.0	3.8	3.0	3.0	0.31
51	AA7f	4	20.7	17.7	3.0	2.3	1.5	3.0	2.8	3.2	0.30
79	AA7f	3	20.5	17.7	3.6	2.1	2.1	2.7	2.7	2.7	0.29
134	AA7f	3	19.9	18.7	3.3	1.4	1.4	2.8	2.7	3.0	0.29
41	AA7d	6	20.4	17.8	3.3	2.5	2.3	3.0	3.0	3.0	0.29
18	AA7d	4	20.2	17.7	3.2	2.0	1.5	3.3	2.8	2.7	0.28
107	AA7f	3	20.3	16.7	3.3	2.8	2.4	3.7	2.7	2.7	0.27
29	AA7d	3	19.8	17.2	3.0	2.0	2.0	3.0	3.0	3.4	0.27
118	AA7f	3	19.7	16.5	3.7	3.0	2.3	3.2	3.0	3.7	0.25
13	AM7e	3	19.0	17.2	3.0	1.1	1.0	3.1	3.3	3.4	0.24
24	AA7d	3	18.7	17.0	3.3	2.4	1.7	2.8	2.7	3.3	0.23
140	AA7h	3	18.3	17.6	3.3	1.1	1.1	3.1	2.4	2.0	0.23
7	AM7e	3	18.7	16.5	3.4	2.3	2.3	3.7	2.7	3.4	0.23
2	AM7b	3	17.8	17.7	3.4	2.9	2.0	3.6	3.3	3.7	0.22
16	AA7d	3	18.1	16.9	3.3	2.8	1.7	3.7	2.6	3.4	0.22
14	AA7d	4	17.4	17.7	3.0	2.1	1.5	3.0	3.2	3.7	0.21
144	AA7h	3	17.0	18.1	3.0	1.0	1.0	2.6	3.0	3.3	0.21
84	AA7f	4	17.6	16.6	3.0	2.0	1.7	2.7	3.0	2.8	0.20
49	AA7f	4	17.4	16.5	3.5	2.6	2.1	3.3	3.3	3.0	0.20
43	AA7f	3	17.7	15.8	3.0	3.0	2.0	3.7	2.6	3.3	0.19
80	AA7f	3	16.7	17.6	3.4	1.3	1.4	2.6	2.3	3.0	0.19
40	AA7d	3	17.1	16.5	3.4	2.6	1.6	2.9	3.0	3.0	0.19
57	AA7f	3	17.2	16.2	3.7	3.0	2.7	3.6	2.7	2.7	0.19
99	AA7f	3	17.2	16.2	3.4	3.0	2.4	2.6	3.0	3.1	0.19
127	AA7f	4	17.2	16.2	3.5	1.7	1.5	3.2	2.5	2.8	0.19
78	AA7f	5	16.7	16.9	3.6	1.8	1.4	3.2	3.2	2.8	0.19
62	AA7f	3	17.1	15.8	3.6	1.1	1.0	3.0	3.7	3.0	0.18
48	AA7f	4	16.5	16.7	3.3	2.5	1.8	2.7	3.1	3.8	0.18
130	AA7f	3	16.4	16.2	3.4	1.9	1.3	2.5	2.3	2.2	0.17
50	AA7f	3	16.3	16.3	3.6	2.1	1.7	3.4	2.4	2.7	0.17
88	AA7f	3	17.1	14.7	3.6	2.0	2.0	3.0	2.3	2.6	0.17
131	AA7f	5	17.0	14.3	3.4	2.2	1.6	3.6	2.8	3.4	0.16
94	AA7f	3	15.8	16.5	3.7	1.0	1.0	3.3	3.0	3.1	0.16
137	AA7h	4	15.5	16.8	3.5	3.0	2.3	3.8	3.3	3.3	0.16
60	AA7f	5	15.8	16.1	3.2	2.6	1.8	2.8	2.8	2.8	0.16
95	AA7f	4	16.1	15.3	3.0	1.5	1.5	3.0	3.0	3.3	0.16
128	AA7f	3	16.0	15.3	2.9	1.0	1.0	2.8	2.9	3.4	0.15
9	AM7e	3	15.9	15.2	3.0	1.8	1.4	3.1	3.0	3.3	0.15
30	AA7d	4	16.2	14.3	3.5	1.0	1.0	3.3	3.5	3.5	0.15
97	AA7f	4	15.1	16.2	4.0	2.7	1.7	2.8	3.2	3.0	0.15
89	AA7f	3	15.4	15.6	3.0	1.7	1.4	3.0	3.1	3.7	0.14
122	AA7f	3	15.4	15.5	3.0	2.1	1.4	2.1	2.9	2.7	0.14
8	AM7e	5	15.2	15.2	3.4	2.2	1.6	3.2	3.0	2.6	0.14
87	AA7f	3	15.2	14.7	3.0	1.2	1.3	3.2	2.3	2.2	0.13
135	AA7f	3	15.1	14.7	3.0	1.4	1.4	3.6	3.0	3.4	0.13
125	AA7f	3	15.8	13.3	3.6	2.0	2.0	4.1	2.9	3.4	0.13
56	AA7f	3	15.0	14.6	3.6	2.1	1.4	3.4	3.0	2.7	0.13
4	AM7b	4	14.4	15.5	3.5	1.8	1.5	2.5	3.0	3.3	0.13
34	AA7d	3	14.9	14.6	3.3	2.1	1.7	3.1	3.0	3.4	0.13
138	AA7h	4	14.8	14.7	3.5	1.5	1.2	2.7	3.0	3.2	0.13
20	AA7d	4	14.4	15.2	3.0	1.7	1.5	2.5	2.5	2.7	0.12
115	AA7f	4	14.4	14.6	3.2	1.8	1.8	3.1	3.2	3.0	0.12
64	AA7f	4	14.1	15.3	3.8	2.2	1.5	3.0	2.5	2.5	0.12
68	AA7f	5	15.2	13.1	3.8	2.2	1.6	3.4	3.0	3.0	0.12
86	AA7f	4	14.4	14.3	3.5	1.7	1.5	3.1	2.7	2.5	0.12
121	AA7f	4	13.8	14.6	3.2	1.3	1.3	2.6	2.5	2.8	0.11
65	AA7f	4	13.7	14.5	3.5	1.2	1.2	2.9	3.3	3.2	0.11
136	AA7f	4	13.6	14.4	3.8	1.4	1.2	3.4	3.0	3.4	0.10
142	AA7h	3	14.6	11.7	3.3	2.0	1.4	3.1	3.0	2.7	0.10
19	AA7d	3	13.4	13.6	3.3	1.0	1.0	3.4	3.0	3.6	0.10
25	AA7d	3	12.7	15.0	3.0	1.9	1.3	3.6	2.6	3.0	0.10
76	AA7f	3	13.3	13.7	3.4	2.0	1.4	3.9	3.0	3.6	0.10
67	AA7f	3	12.9	14.0	3.6	1.1	1.0	3.4	2.7	3.7	0.09
36	AA7d	4	12.9	13.3	3.3	1.2	1.2	3.2	2.7	3.3	0.09
33	AA7d	3	12.9	13.0	3.4	1.6	1.3	2.7	2.7	3.1	0.09
105	AA7f	3	13.0	12.9	3.4	0.9	1.0	2.3	2.6	3.3	0.08
133	AA7f	4	12.3	14.1	3.5	2.1	1.5	3.5	2.2	3.0	0.08
45	AA7f	3	11.6	14.2	3.0	1.1	1.0	3.3	3.0	2.9	0.07
141	AA7h	3	11.2	14.0	3.3	2.1	1.4	2.8	2.7	3.8	0.07
31	AA7d	4	12.2	11.8	3.5	2.8	2.3	3.3	3.2	3.2	0.07
129	AA7f	3	10.7	13.8	4.0	1.6	1.3	3.6	3.3	3.3	0.06
117	AA7f	3	11.0	10.9	3.0	1.7	1.3	3.4	3.0	2.4	0.05
54	AA7f	3	9.2	10.5	3.6	1.1	1.0	2.3	2.3	2.6	0.03

Selection of site-specific or general performer families in *Acacia crassicarpa* trials (Brumas and Luasong)

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Introduction

Acacia crassicarpa is a fast growing tree species that is sometime preferred to *A. mangium* because it has a thin bark that can easily be peeled off by a debarker. Its wood is also slightly denser, so that the general yield in pulpwood is believed to be higher as compared to *A. mangium*.

A new contract has recently been established between the Plant Biotechnology Laboratory (ICSB / CIRAD-Foret) and Sabah Softwood Sdn. Bhd. to propagate some *A. crassicarpa* materials by tissue culture. The objective of the present work was to accompany the propagation work of the laboratory with an accurate analysis of the genetic value of the available *A. crassicarpa* materials.

Several progeny trials have been established in the past with the same families of *Acacia crassicarpa*, three in Luasong (SSO1, SS02 and SSO3) and one in Brumas (AC20a). A joint statistical analysis of SSO1, SSO2 and AC20a was carried out, and the genetics of these families in different environments was studied. A selection of genetic materials with good performances over the different sites was also prepared and compared to a mono-site selection.

Material and methods

In the late 80s, an expedition to Papuan New Guinea and Northern Australia was organised in order to collect seeds from natural stands of *Acacia crassicarpa*. A number of seedlots were collected on unrelated single plus trees; these seedlots genetically correspond to open-pollinated progenies. Few seedlots were made up of a bulk of progenies, thus corresponding to "provenances". Over sixty progenies and four bulks (provenances) were collected in few locations of Papua New Guinea, all situated in a flat and flood-prone area of about 100 km of diameter. One additional seedlot was collected as a bulk of progenies (provenance) from Olive River, in Queensland (Australia).

The seeds were distributed by ACIAR both to Luasong and Brumas. In Luasong, the material, after germination, was planted in the field in April 1990, in two complete randomised block designs, SSO1 and SSO2, of 0.96 and 1.05 ha and with 4 and 5 repetitions respectively. The experimental unit included 5 trees planted in a single row. SSO1, established in a flat area near a river formerly occupied by natural forest, included 61 progenies and 3 bulks, all from 8 provenances in Papua New Guinea. SSO2, located near SSO1 but on a higher ground, included 52 progenies and four bulks from the same 8 provenances. The Luasong trials, planted at a 2.5 x 3 m spacing, have been selectively thinned twice, a first time in October 1991, taking away 3 trees out

of 5 for each experimental unit, and a second time in January 1993, taking away one out of 2 trees per experimental unit.

In Brumas, the material was planted in April 1995 on an area of 3.38 ha, in a complete randomised block design with six repetitions (AC20a). The area is on a slope and was formerly planted with other Acacia species. The spacing was 3 x 3 m and the experimental unit contained 10 trees in a double row. The material planted in AC20a included 55 progenies and 8 bulks from 11 provenances. The three additional provenances of AC20a included one provenance from Queensland (Olive River) and two from the same area of the other materials of PNG. No thinnings were carried out on this trial.

All trials have been assessed every year since planting. For our common analysis, we chose to study the assessment of 1998 (two years and eleven months after planting) for AC20a, and the assessment of May 1992 for the two Luasong trials (two years and one month after planting). The data of the assessment of May 1993 for Luasong could not be used because the second thinning had already taken place, leaving only one tree per experimental unit.

The measured characters were :

- Mortality (scores: 0=alive, 1=dead)
- Diameter at the breast height (in centimeters)
- Height (in meters)
- Straightness (scores from 1=straight to 6=extremely crooked)
- Fork (scores: 1=no fork; 2=one fork in the upper portion of the trunk; 3=one fork in the medium portion of the trunk; 4=one fork in the lower portion of the trunk)

Firstly, a global analysis was carried out on a database containing the three trials. The model for this analysis was as follows:

1) *single tree measurement = site effect + repetition within site effect + provenance effect + progeny within provenance effect + site * prov. interaction + site * fam. within prov. interaction + rep. within site * prov. interaction + error*

For this analysis, we had to discard all the families and provenances that were not represented in all the three trials. In order to have enough within-treatment variance for the analysis, we also discarded: i) repetition 6 in AC20a, that had problems at planting (not enough materials), ii) all the experimental units with less than two living trees, and iii) all provenances with less than two families. The *fam.*rep. within prov.*site interaction*, that is the environmental effect of the experimental unit, was not taken in account, because there was no reason to believe that the only two trees in each experimental unit of the two trials in Luasong represented the true genetic value of the family.

Secondly, additional analyses were carried out with modified models: 1) an analysis without the provenance effect, allowing us to reintroduce in the analysis all materials with less than two families per provenance that were discarded in the previous analysis; 2) pairwise comparisons of couple of sites; 3) an analysis where all interactions were transferred in the error term.

Thirdly, one separate analysis of variance were carried out on the Brumas trial, according to the following model:

2) *single tree measurement = repetition effect + provenance effect + progeny within provenance effect + interactions with repetition + error*

All main effects were tested on the appropriate interactions. These analysis allowed to make a comparison of the selection carried out on a single trial or globally on the three trials.

Genetic parameters (averages corrected for the model, heritability, genetic value of a family and genetic gain), were calculated as indicated in Appendix 1. In addition, the genetic value of the family in the poorest site, its reactivity and the accuracy of this prediction were calculated according to the regression theory, where the mean family values in each site were regressed against the general mean of each site; the three parameters correspond to the intercept, the slope and the standard deviation of the regression line respectively.

Results

Multi-site analysis

- *Provenance effect*

The multi-site analysis for this level of variation included 40 families from 4 provenances, all present in the tree trials with at least two trees per experimental unit and two families per provenance.

The analysis showed that the provenance effect was generally not significant. In fact, there was a large unbalance between provenances, 2 provenances being represented by 16 and 17 families, and the remaining 6 by only 2 to 4 families. The provenance effect remained not significant even taking in account only the two largest provenances. This fact, in addition to the geographical proximity among populations (the only seedlot collected in Queensland was excluded from the analysis because confounding provenance and family levels), authorised us to delete the provenance effect from the analysis. From here onward, the provenance effect was discarded from the statistical model, and four families formerly discarded were reintroduced in the dataset.

- *Site effect*

The site effect was significant for all characters, especially the diameter, the fork and the straightness, for which it absorbed more than 35% of the total variance (Table 1). For the height, however, the site effect accounted only for 4% of the variance. Diameter and trunk form were all heavily influenced by the different treatments given to the trials in Luasong and in Brumas, the former being planted at a closer spacing but selectively thinned. As expected, the height was less affected by the site effect and the silviculture treatments; this trait is often considered as an indicator of the site quality. Based on height, the SSO1 appeared to be the best site, and the difference becomes more noticeable taking in account the shorter time passed between planting and assessment in this trial as compared to AC20a. The average values per character and per site are given in Table 2.

Table 2. Average values of the characters in the three sites.

Site	Height	Diameter	Fork	Straightness
SSO1	10.57	9.77	4.45	2.02
SSO2	9.34	8.52	4.30	2.17
AC20a	9.63	12.76	2.01	3.67

Table 1. Global analysis of variance over the three trials.

	Source of variation	Degrees of Freedom	Mean Square	Value of the F test	Probability of error	Component of Variance
Height	*Model*	481	5.60	2.13	0.0001	
	SITE	2	80.73	14.78	***0.0001***	5%
	REP(SITE)	11	18.31	4.52	***0.0001***	8%
	FAMILY	43	7.21	1.78	***0.0028***	4%
	*SITE*FAMILY*	86	5.46	2.08	***0.0001***	1%
	*FAMILY*REP(SITE)*	339	4.05	1.54	***0.0001***	11%
	Error	757	2.63			71%
Diameter	*Model*	481	22.00	2.01	0.0001	
	SITE	2	1910.73	159.74	***0.0001***	38%
	REP(SITE)	11	24.02	2.05	***0.0234***	2%
	FAMILY	43	10.22	0.87	0.6994	0%
	*SITE*FAMILY*	86	11.96	1.09	0.2765	4%
	*FAMILY*REP(SITE)*	339	11.71	1.07	0.2312	3%
	Error	757	10.96			52%
Fork	*Model*	481	4.36	4.03	0.0001	
	SITE	2	653.00	681.45	***0.0001***	37%
	REP(SITE)	11	1.95	2.04	***0.0239***	1%
	FAMILY	43	0.92	0.97	0.5362	1%
	*SITE*FAMILY*	86	0.96	0.89	0.7582	0%
	*FAMILY*REP(SITE)*	339	0.95	0.88	0.9124	8%
	Error	757	1.08			57%
Straightness	*Model*	481	2.27	5.20	0.0001	
	SITE	2	290.63	473.28	***0.0001***	76%
	REP(SITE)	11	1.29	2.07	***0.0218***	1%
	FAMILY	43	0.76	1.22	0.1699	2%
	*SITE*FAMILY*	86	0.61	1.41	***0.0118***	0%
	*FAMILY*REP(SITE)*	339	0.62	1.43	***0.0001***	2%
	Error	757	0.44			19%

Note: SITE=site effect; REP(SITE)= repetition within site effect; FAMILY=family effect; SITE*FAMILY= interaction of site with family; FAMILY*SITE(REP)=interaction of family with repetition within site

A partition of the site effect according to couple of sites (Table 3) showed that SSO1 and SSO2 were different for the height, and hence probably for the fertility (SSO2 being located on higher ground as compared to SS01), but not so much for diameter and characters of trunk form (maybe because of the same thinning regime); while the two Luasong trials differed from the Brumas' one for all characters more depending on the thinning regime.

Table 3. Percentage of variance due to site in pairwise comparisons of sites.

	Height	Diameter	Fork	Straight.
SSO1-SSO2	16%	10%	3%	1%
SSO2-AC20a	0.6%	43%	32%	70%
SSO1-AC20a	9%	27%	48%	73%

- Repetition within site effect

The repetition effect within sites was also significant for all characters (Table 1), translating the dependence of the development of the trees on the environment. This was a positive finding, because repetitions are supposed by construction to absorb as much as possible of environmental variation. In fact, the character that showed a stronger repetition effect was the height (8%), confirming its dependence on the environment.

- Family effect

The family effect was significant only for the height, contributing in this case to 4% of the total variation. For the other characters, the differences between sites and repetitions absorbed most of the variation, while the relatively small number of trees per family in Luasong (7.5 and 8.5 on average in SSO1 and SSO2 respectively) probably played a role hiding this effect.

- Interaction of family with site

A significant family per site interaction effect means that some families are better adapted to one site than to another, or in other words, that a family selected in one site will not perform so well in another site. In the global analysis, this effect was found for height and straightness. However, the part of variance explained by the interaction was small if compared to the main family effect, leaving enough variation to carry out a multi-site selection.

The three sites differed from each other not only for the silviculture, but also for the soil conditions, AC20a being on a slope, SSO1 being rather on a flat area near a river, and SSO2 near to SSO1 but on higher ground; also, prior to planting, the Luasong trials were occupied by a natural forest, which usually bring along a number of biologically active soil components. The last element of difference was the time; the assessment of the Luasong trials covered the period 1990 to 1992, while in AC20a it covered from 1995 to 1998. Dry or wet years alternate frequently in Sabah, maybe explaining part of the differences. Given the natural distribution of *A. crassicarpa* that essentially cover wet regions, some families may have developped an adaptation to humid sites, while others to drier soils. However, due to the large differences in family performance found between SSO1 and SSO2 (Table 4) especially for diameter and straightness, this hypothesis may be regarded critically.

Two alternative hypotheses that could explain this interaction are: 1) that local environmental heterogeneity is very large, and the average of a family in one site essentially reflects differences in

fertility within site; 2) that genetic variability is very large, and the 7-8 trees per family in Luasong did not represent the true family value.

Table 4. Percentage of variance due to interaction site*family in pairwise comparisons of sites.

	Height	Diameter	Fork	Straight.
SSO1-SSO2	1%	7%	1%	15%
SSO2-AC20a	0.5%	1%	0.4%	0.5%
SSO1-AC20a	3%	4%	0.5%	0.2%

- Interaction of family with repetition within site

As stated in the material and methods, this interaction, that should represent the environmental effect of the experimental unit, may generate problems to the analysis, since the experimental units were composed of 2, 2 and 3.7 trees on the average in SSO1, SSO2 and AC20a respectively. With so few trees, there was no reason to believe that the true genetic value of the family was really represented in the experimental unit. However, the analysis gave the same pattern either including this effect in the model, or putting it in the experimental error. We chosed to show this effect in Table 1 because its large contribution to the total variance (11% in the case of the height) may help to understand the weight of mixed environmental and genetic effects on small samples.

- Multi-site ranking for selection

Genetic values for each family in the multi-site analysis are presented in Table 5. The least square mean represents the mean for the family over the tree sites, adjusted for the model (i.e. taking in account the site, repetition and the other effects). The genetic value was calculated by weighting the difference between mean of the family and the general mean by the contribution of families to the total phenotypic variance. The value of the family in the poorest site and its reactivity are the intercept and slope of the regression among the value of the family in each site and the average value for each site.

Biologically, the table can be read as follows: family n. 1096 is the best of all, but it performs not well in the poorest site: it is a "site-specialist". The error attached to its reactivity prediction is relatively small (CV<60%). Family 1111 and 1172 performs well in all sites, especially in the poorest. They are "generalists". However in their case the error is higher and there is a possibility of mistake in a multisite selection. Family 1169 behave like 1096, but in this case the probability of error is very low; selection of this family for the best sites is reliable. Families 1108 and 1256 are the first two generalists with a relatively low probability of error. And so on.

Other tables like n. 5 can be prepared for the other characters, which display however less significant differences among families; selection will be less reliable in those cases. The weight to give to each character in the selection is a choice depending also on their economic value.

- Heritability and Genetic gains

The genetic gain that is possible to achieve through selection was also calculated, based on heritability (Table 6). Narrow sense heritability was 0.33 for the tree height, which being calculated over three site should be considered a good result.

Table 5. Genetic parameters for the growth in height according to families, ranked by their genetic value.

Family	Least Square Mean	Standard Error	Genetic Value	Value in the poorest site	Reactivity	Standard error	Probability of error
1096	11.01	0.37	0.62	-1.72	1.30	0.78	0.34
1111	10.79	0.35	0.51	7.59	0.32	1.26	0.84
1172	10.72	0.39	0.48	4.16	0.69	1.15	0.66
1169	10.70	0.40	0.47	-3.67	1.45	0.08	0.03
1268	10.66	0.43	0.45	17.47	-0.34	0.26	0.21
1272	10.60	0.39	0.42	-3.08	1.37	0.94	0.38
1108	10.54	0.32	0.39	3.68	0.70	0.36	0.31
1256	10.48	0.39	0.36	6.43	0.41	0.13	0.19
1168	10.44	0.39	0.34	-3.19	1.38	0.07	0.03
1185	10.42	0.35	0.33	5.77	0.47	1.34	0.79
1102	10.41	0.36	0.32	1.52	0.86	0.60	0.39
1166	10.31	0.35	0.27	10.40	-0.01	0.29	0.97
762	10.27	0.41	0.25	-2.66	1.25	0.05	0.03
1151	10.25	0.55	0.24	-5.80	1.64	0.23	0.09
1110	10.23	0.38	0.23	-13.26	2.35	0.15	0.04
1270	10.20	0.42	0.22	-2.47	1.25	2.27	0.68
1100	10.05	0.44	0.15	-7.75	1.78	0.65	0.22
1127	10.01	0.44	0.12	-1.39	1.16	0.01	0.01
1131	9.98	0.34	0.11	-11.38	2.17	0.89	0.25
1255	9.97	0.33	0.11	4.38	0.56	0.88	0.64
1263	9.96	0.39	0.10	-5.79	1.57	0.63	0.24
1254	9.95	0.45	0.10	0.28	1.00	0.76	0.41
1115	9.92	0.41	0.08	-10.02	1.97	0.77	0.24
1105	9.88	0.45	0.06	-2.67	1.22	0.49	0.24
1165	9.88	0.34	0.06	14.09	-0.41	0.03	0.05
1124	9.79	0.35	0.02	-6.28	1.61	1.00	0.35
1171	9.68	0.39	-0.04	-2.36	1.22	0.12	0.06
1183	9.66	0.42	-0.05	-2.73	1.24	1.09	0.46
1125	9.66	0.36	-0.05	9.80	0.00	0.38	1.00
1175	9.61	0.46	-0.07	1.56	0.82	0.95	0.55
1182	9.60	0.44	-0.08	4.20	0.54	0.65	0.56
1174	9.56	0.38	-0.10	-0.95	1.09	0.21	0.12
1138	9.50	0.40	-0.13	4.84	0.52	0.38	0.40
1173	9.39	0.44	-0.18	8.15	0.18	1.76	0.94
1253	9.39	0.35	-0.19	9.11	-0.01	0.85	0.99
1170	9.22	0.42	-0.27	-13.69	2.35	1.56	0.37
1167	9.21	0.40	-0.28	-4.33	1.37	0.79	0.33
1184	9.07	0.42	-0.34	-5.12	1.39	0.69	0.29
1179	8.75	0.44	-0.50	-9.86	1.95	0.87	0.27
1113	8.49	0.34	-0.63	-12.14	2.10	0.72	0.21
1106	8.25	0.38	-0.75	18.48	-1.02	1.78	0.67

Note: Reactivity >1: families with good performances in the best sites but bad in poor sites; 0 < Reactivty < 1: families with little reactivity, doing homogeneously in rich or poor sites; Reactivity = 0: completely stable families in relation to environment; Reactivity<0: a biological non-sense (a family performs better in poor sites than in good sites), it is generated by errors of the model (due to small number of trees per family for example).

Table 6. Heritability value of height in the multi-site analysis.

Variable	Average	Mean Square Family	Mean Square error	Average number of trees in the family	Family variance	Narrow sense heritability	Broad sense heritability	Prob. error
Height (m)	9.69	7.21	2.63	19.30	0.24	0.33	0.64	0.003

The genetic gain is then derived from heritability, taking in account that, in open-pollination families, ¼ of the genetic value of the mother tree is passed on to the progeny. In case controlled crosses could be realised, then the genetic gain would be higher.

By collecting and planting seeds of the best ten trees over the three sites, the genetic gain realised in plantation should be 15.9% (Table 7). A similar results can be obtained selecting the best 3 trees out of the best 3 families (this value could actually be higher, would we had a more advanced genetics software like those based on the "Best Linear Unbiased Prediction Model"; CIRAD-Foret is at present working to its development).

What is worth noting here is that this selection and the relative genetic gain is valable over the three sites and probably in most of the Luasong-Brumas area.

Table 7. Genetic gains obtained through phenotypic or combined individual / family selection in the multi-site analysis. Character: height.

	PHENOTYPIC SELECTION		COMBINED SELECTION			
Family Selection			Ind / Fam 40% / 2.5%	Ind / Fam 7% / 14%	Ind / Fam 14% / 7%	Ind / Fam 40% / 25%
Total selection pressure	1%	10%	1%	1%	1%	10%
Number of selected trees	10	100	8 individuals from 1 family	1 individual from 8 families	3 individuals from 3 families	8 individuals from 10 families
	PHENOTYPIC SELECTION		COMBINED SELECTION			
Genetic gain	***15.9%***	***10.5%***	***13.5%***	***13.6%***	***14.6%***	***9.6%***

Separate analysis

- Mortality

The trials in Luasong were thinned before the assessment, so that the study of mortality has no sense there. By contrast, in AC20a, where no thinnings were carried out, a mortality of 40% was observed in the first year after planting, and an additional 16% of the trees died between year 1 and 3. The statistical analysis showed that significant differences in survival existed among repetitions (with a probability of error of P=0.01), but not among families nor provenances. The ranking of the families in terms of survival is given in Table 8. It is worth noting that the best survivors in AC20a(1168, 1272, 1169 and 1268) ranked also among the firsts in the multi-site analysis.

Table 8. Ranking for survival in AC20a.

Rank	Family	Survival	Rank	Family	Survival
1	1168	0.66	25	1253	0.36
2	1272	0.60	26	1115	0.34
3	1169	0.54	27	1133	0.34
4	1268	0.54	28	1167	0.34
5	1106	0.50	29	1173	0.34
6	1104	0.46	30	1179	0.34
7	1105	0.46	31	1183	0.34
8	1125	0.45	32	1100	0.33
9	1131	0.45	33	1170	0.33
10	1166	0.43	34	1175	0.32
11	1096	0.42	35	1113	0.30
12	1111	0.42	36	1127	0.30
13	1124	0.42	37	1252	0.30
14	1256	0.42	38	1117	0.28
15	1263	0.42	39	1185	0.28
16	1108	0.40	40	1171	0.27
17	1128	0.40	41	1110	0.26
18	1102	0.38	42	762	0.25
19	1174	0.38	43	1095	0.25
20	1255	0.38	44	1112	0.25
21	1165	0.38	45	1172	0.25
22	782	0.36	46	1270	0.25
23	1138	0.36	47	1184	0.23
24	1182	0.36	48	768	0.22
			49	1254	0.15

- Growth

The analysis of variance of the trial AC20a raised the same problem than the global analysis, i.e. that the interaction repetition*family, based on a small number of trees (3.8), brought a decrease in the contribution of the family effect. We decided then to discard again this effect. The analysis of the main effects showed a significant family effect for 3 out of 4 characters, namely height, diameter and straightness (Table 9).

The heritability and genetic gain achievable for these traits with comparable selection pressure than those applied above (Table 7) are shown in Table 10 and 11 respectively. The characters that are more heritable are the height, the straightness and the fork, in that order.

Table 10. Heritability for several characters in the trial AC20a.

Variable	Average	Mean Square family	Mean Square error	Average number of trees in the family	Family variance	Narrow sense heritability	Broad sense heritability	Prob. error
Height (m)	9.68	6.67	2.62	14.14	0.29	***0.39***	0.61	***0.00***
Diameter (cm)	12.92	18.18	12.45	14.14	0.41	***0.13***	0.32	***0.02***
Fork	2.07	1.33	1.21	14.14	0.01	0.03	0.09	0.45
Straightness	3.68	0.75	0.40	14.14	0.02	***0.23***	0.47	***0.01***

Table 9. Analysis of variance for the Brumas trial.

	Source of variation	Degrees of Freedom	Mean Square	Value of the F test	Probability of error	Component of Variance
Height	*Model*	60	11.13	3.87	***0.0001***	
	REPETITION	4	68.02	23.66	***0.0001***	12%
	FAMILY	56	6.70	2.33	***0.0001***	6%
	Error	834	2.87			82%
Diameter	*Model*	60	23.16	1.78	***0.0004***	
	REPETITION	4	77.03	5.91	***0.0001***	3%
	FAMILY	56	18.60	1.43	***0.0242***	2%
	Error	834	13.04			95%
Forking	*Model*	60	1.60	1.26	0.0968	
	REPETITION	4	5.99	4.71	***0.0009***	2%
	FAMILY	56	1.29	1.02	0.4459	0%
	Error	834	1.27			98%
Straightness	*Model*	60	0.79	1.74	***0.0006***	
	REPETITION	4	2.60	5.71	***0.0002***	2%
	FAMILY	56	0.67	1.48	***0.0147***	3%
	Error	834	0.46			95%

Table 11. Genetic gains obtained through phenotypic or combined individual / family selection in AC20a, Brumas.

	PHENOTYPIC SELECTION		COMBINED SELECTION			
Family Selection			Ind / Fam 40% / 2.5%	Ind / Fam 7% / 14%	Ind / Fam 14% / 7%	Ind / Fam 40% / 25%
Total selection pressure	1%	10%	1%	1%	1%	10%
Number of selected trees	10	100	8 individuals from 1 family	1 individual from 8 families	3 individuals from 3 families	8 individuals from 10 families
Genetic gains	**PHENOTYPIC SELECTION**		**COMBINED SELECTION**			
Height	***18.6%***	***12.4%***	***17.3%***	***16.6%***	***18.3%***	***12.2%***
Diameter	***9.4%***	***6.2%***	***10.8%***	***9.9%***	***11.2%***	***7.5%***
Fork	***4.0%***	***2.6%***	***5.1%***	***4.7%***	***5.3%***	***3.6%***
Straight.	***11.1%***	***7.3%***	***11.5%***	***10.8%***	***12.1%***	***8.1%***

As expected, the genetic gains in trial AC20a were slightly higher than those in the multi-site selection scheme; however the multi-site selection is largely more robust, as its results are applicable on a larger range of sites.

This can be seen by comparing the ranking for the height, the most heritable character, in AC20a and in the multi-site analysis (Table 12). Many trees change considerably of rank, as for exampe family 1255, that would be selected in Brumas but not on a multi-site basis, or families 1111 and 1272 that have a great value in the multi-site selection but not in AC20a.

Conclusion

This exercise was an example of the advantage of having multi-site trials, which consists mainly in the possibility to prepare either a unique selection of trees adapted to a large range of sites, or of more specialised materials each adapted to a particular site condition.

In spite of the fact that the multi-site experiment was not planned *a priori* and, in the present conditions, had a number of drawbacks (such as different planting dates and different silviculture treatments in Luasong and Brumas), the multi-site analysis brought about a large quantity of new information concerning the stability of the families in different sites, and the effect of the environment. It also allowed to prepare several selections, the first one of "generalist" families, more robust and "safe", the second of families performing well in poor sites, and the third with the best genotypes for the best environment.

Considering the height, the five best families in AC20a have an average genetic value (in the multi-site analysis) of 0.34, while this value for the best five families in the multi-site analysis was 0.50. In a multi-site environment, and with a selection pressure of 10%, the multi-site selection was then 30% better than the selection done on AC20a alone. The genetic gains for height should be around 15 to 18 %, while for diameter one can hope, in plantingsless affected by mortality, to be

able to achieve a gain of 12%. The genetic gains for diameter and height usually combine together, in this case giving a probable genetic gain of more than 30% with a 1% selection coefficient. A selection coefficient of 1% is reasonable giving that the base population is composed of more than 1400 trees.

Once again, the weight to give to the different characters or to the ranking in AC20a as compared to the global ranking depends on economic and ecologic criteria. For example, are all the sites in Brumas similar to AC20a (on a slope, etc.) or are there some areas more similar to the environment in SSO1 (flat and humid) or SSO2 (flat on the top of a hill) ?

Appendix 1

GENETIC FORMULAE

Family variance at the trial level :

$$\sigma^2_{fam} = \frac{MeanSquare_{fam} - MeanSquare_{error}}{n}$$

Phenotypic variance at the trial level :

$$\sigma^2_P = \sigma^2_{error} + \sigma^2_{fam} = MeanSquare_{error} + \sigma^2_{fam}$$

Prediction of the genetic value of one family *i* :

$$\hat{F}_i = \frac{\sigma^2_{fam}}{\text{var}\, P_i}(P_i - \mu)$$

Average heritability at the families level (half-sib open-pollinated families) :

$$h^2_{narrowsense} = \frac{\sigma^2_A}{\sigma^2_P} = \frac{4 * \sigma^2_{fam}}{\sigma^2_P} \qquad h^2_{broadsense} = \frac{\sigma^2_{fam}}{\sigma^2_{fam} + \frac{MS_e}{EMS}}$$

Table 12. Family values for several characters, ranked by height, for the Brumas trial AC20a. In the column at the left, the rank value of the families in the multi-site analysis is displayed for comparison with the ranking in Brumas.

Multi-site rank	Brumas rank	family	height	standard error	diameter	standard error	fork	standard error	straight.	standard error
3	1	1172	11.55	0.60	13.88	1.29	1.67	0.40	3.13	0.24
10	2	1185	11.24	0.54	13.06	1.15	1.58	0.36	3.58	0.21
8	3	1256	10.50	0.37	14.72	0.79	1.97	0.25	3.40	0.15
20	4	1255	10.42	0.40	13.97	0.86	2.19	0.27	3.70	0.16
4	5	1169	10.34	0.33	14.30	0.70	1.99	0.22	3.79	0.13
11	6	1102	10.27	0.40	13.05	0.85	1.88	0.27	3.83	0.16
1	7	1096	10.25	0.37	13.46	0.79	2.09	0.25	3.61	0.15
14	8	1151	10.22	0.37	13.66	0.79	2.73	0.25	4.22	0.15
30	9	1175	10.15	0.46	13.11	0.97	2.30	0.30	3.64	0.18
5	10	1268	10.15	0.33	11.89	0.70	2.04	0.22	3.64	0.13
7	11	1108	10.14	0.38	13.72	0.81	1.95	0.25	3.75	0.15
9	12	1168	10.12	0.30	13.45	0.63	2.07	0.20	3.62	0.12
29	13	1125	10.10	0.42	14.47	0.89	2.58	0.28	3.81	0.17
--	14	1252	10.10	0.51	13.11	1.09	1.46	0.34	3.74	0.20
36	15	1170	10.07	0.54	13.95	1.15	1.64	0.36	3.79	0.21
25	16	1165	10.06	0.44	13.78	0.94	2.26	0.29	3.87	0.18
33	17	1138	10.05	0.41	14.15	0.88	2.41	0.27	3.79	0.16
12	18	1166	10.00	0.47	12.58	1.01	1.84	0.31	3.83	0.19
41	19	1106	9.92	0.38	13.55	0.81	2.48	0.25	3.58	0.15
18	20	1127	9.88	0.51	12.53	1.10	1.65	0.34	3.27	0.20
17	21	1100	9.86	0.57	14.43	1.21	2.29	0.38	3.84	0.23
21	22	1263	9.85	0.37	12.94	0.79	1.69	0.25	3.58	0.15
--	23	1104	9.80	0.35	12.95	0.75	2.34	0.24	3.72	0.14
2	24	1111	9.75	0.37	13.05	0.79	2.12	0.25	3.74	0.15
--	25	782	9.74	0.40	12.41	0.85	1.95	0.27	3.72	0.16
--	26	1117	9.73	0.57	11.50	1.22	1.71	0.38	3.59	0.23
32	27	1174	9.73	0.40	13.25	0.86	2.10	0.27	3.85	0.16
--	28	1133	9.71	0.43	12.34	0.91	2.22	0.28	3.38	0.17
35	29	1253	9.60	0.41	13.11	0.88	1.95	0.27	3.99	0.16
23	30	1115	9.60	0.42	13.21	0.90	1.69	0.28	3.30	0.17
--	31	1271	9.58	0.47	13.02	1.01	1.61	0.31	3.82	0.19
--	32	1269	9.51	0.43	13.28	0.91	1.94	0.28	3.80	0.17
24	33	1105	9.47	0.36	13.44	0.76	1.77	0.24	3.46	0.14
22	34	1254	9.45	0.85	10.58	1.81	2.22	0.57	4.07	0.34
6	35	1272	9.44	0.35	12.55	0.74	1.83	0.23	3.33	0.14
--	36	1283	9.43	0.45	11.38	0.97	1.89	0.30	3.26	0.18
--	37	1126	9.37	0.51	12.15	1.09	1.63	0.34	3.88	0.20
13	38	762	9.36	0.61	13.18	1.29	1.58	0.40	3.61	0.24
27	39	1171	9.30	0.60	12.54	1.28	2.21	0.40	3.46	0.24
--	40	1107	9.28	0.46	12.59	0.98	2.52	0.31	3.84	0.18
15	41	1110	9.28	0.50	11.52	1.06	1.87	0.33	3.75	0.20
--	42	1128	9.25	0.60	10.90	1.29	2.54	0.40	3.88	0.24
--	43	1129	9.02	0.54	14.10	1.15	2.11	0.36	4.22	0.22
19	44	1131	8.92	0.40	11.37	0.86	2.20	0.27	3.54	0.16
31	45	1182	8.89	0.40	13.26	0.85	2.12	0.27	3.60	0.16
38	46	1184	8.77	0.60	10.97	1.28	1.66	0.40	3.69	0.24
40	47	1113	8.61	0.44	10.92	0.93	2.03	0.29	3.60	0.17
34	48	1173	8.54	0.44	10.40	0.94	2.09	0.29	3.62	0.18
--	49	768	8.51	0.57	10.35	1.21	1.63	0.38	3.84	0.23
26	50	1124	8.48	0.38	11.27	0.81	1.90	0.25	3.70	0.15
28	51	1183	8.42	0.43	11.59	0.91	1.72	0.28	3.82	0.17
37	52	1167	8.39	0.43	12.05	0.91	2.57	0.28	3.76	0.17
39	53	1179	8.31	0.44	10.62	0.94	1.95	0.29	3.80	0.18
16	54	1270	7.90	0.60	11.12	1.29	1.78	0.40	3.73	0.24

Genetic gain by family selection :

$$\Delta P = \frac{i * h^2_{narrowsenss} * \sqrt{\sigma^2_{fam} * \sigma^2_{error}}}{Exp(x)}$$

Genetic gain by family / individual combined selection :

$$\Delta P = \left[\frac{i_{betweenfam} * 2 * 1/8 * 4\sigma^2_{fam} * (n+3)/n}{\sqrt{\sigma^2_{fam} + MeanSquare_{error} / EMS}} + \frac{i_{withinfam} * 3/8 * 4\sigma^2_{fam} \sqrt{(n-1)/n}}{\sqrt{MeanSquare_{error}}} \right] / Exp(x)$$

SYMBOLS

σ=variance

fam=progeny or family level

Pi..=family mean at the trial level

n=Number of individuals for the Expected Mean Square (harmonic mean)

ΔP=genetic gain

i=selection pressure

E(x)=Expected mean

Appendix 6

Calamus caesius yield plots. Estimation of the length of mature cane.

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Introduction

The *Calamus caesius* yield plots were planted between 1989 and 1991. Sixteen permanent yield plots were planted with the usual line planting. Results of the last assessment, made in October 1997, and prospects of the possible revenue at harvesting are given in this paper.

Material and method.

The yield plots were planted with an original density of 660 plants per hectare. Characters recorded during the previous assessments from 1992 to 1995 were the number of stems and the length of each stem. The length was estimated by the method of Stockdale & Power (1994) when the stem was too long to be measured with a conventional tape meter : the accessible bottom part of the stem was measured with the tape, and the length of the "out of reach" upper part estimated by counting the number of nodes, then multiplying by the mean internode length calculated on the bottom part. Since we found a high correlation between the total length of the plant and the number of stems, in the last assessment in 1997 we recorded only the number of stems per clump. With regression methods, we then used the data of the previous assessment of 1995 to calculate the total length of the clumps in 1997 according to the number of stems.

A model of growth was computed based on the total length of the plants for all the assessments (1992 to 1997). The optimal harvesting date was estimated according to the shape of the curve, when the mean annual increment matches the current annual increment. We then calculated the total length expected at the harvesting time.

An estimation of the length of mature cane per hectare in 1997 was calculated based on the data for *C. caesius* previously recorded by Alloysius (1997) in a 6-year old plot in LFC. In this data set, the length and percentage of mature cane was given according to the total length and the age of the stems. The correlation (R^2) between the age and the percentage of mature cane was w 0.56, and between the length and the percentage of mature cane 0.72. Using the Alloysius' formulas (1997), we envisaged two scenarios for the calculation of the expected income in 1997 in the *C. caesius* yield plots :

- The first scenario estimates the percentage of mature cane according to the age of the plot. Thus, all the canes of one clump were attributed the same age (age of the plot).
- The second scenario estimates the percentage of mature cane according to the estimated mean length of the stems. The conversion index from the length of mature cane to the income was given by Alloysius (36,000 meters for 2,500 RM).

Finally, an estimation of the length of mature cane at harvesting time and the expected income was made following the process described hereafter :

- The estimation of the total length of the clumps per plot in 1997 was used to calculate the expected total length at harvesting time according to the model of growth previously computed.
- The relation between the length of the stem and the percentage of mature cane was applied to the mean average length of the stem computed from the total length hereabove.
- The expected income was then calculated using the same conversion index as for scenario 2 in 1997.

Results and analysis

Results of the assessment in 1997.

Table 1 gives the results of the last assessment made in 1997 : average number of stems, average length of the stems and the mortality. The heterogeneity between the plots and even within the plots was high, some plants over 30 meters being close to small plants still under 1 meter long.

Table 1 : Average number of stems, average, maximum and minimum length of the plant per plot (in m), and survival recorded in the yield plots.

Plot	Avg Nb stems	Avg lgth	Nb dead	% dead
1	5	23.62	1	3%
3	6	48.69	4	13%
4	5	45.19	7	23%
5	21	131.78	3	10%
6	19	86.10	9	30%
8	8	38.16	11	37%
9	8	33.49	2	7%
10	7	45.60	0	0%
11	14	63.80	1	3%
12	4	23.02	9	30%
13	3	7.72	7	23%
14	7	24.04	2	7%
15	2	3.33	6	20%
16	13	45.60	3	10%
18	6	30.27	7	23%
19	1	1.35	11	37%

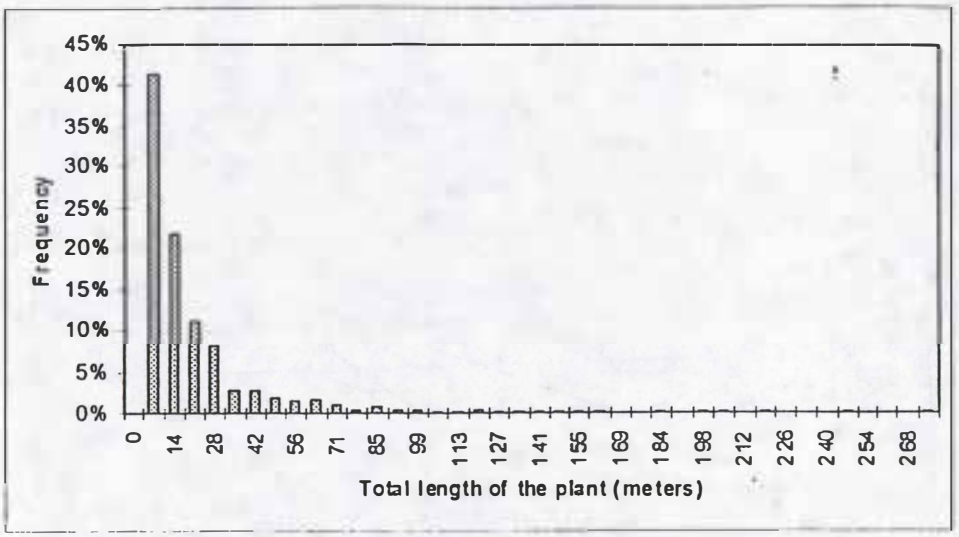


Figure 1 : Distribution of the total length of the plants for the 16 plots.

Comparison of the growth to the previous assessments

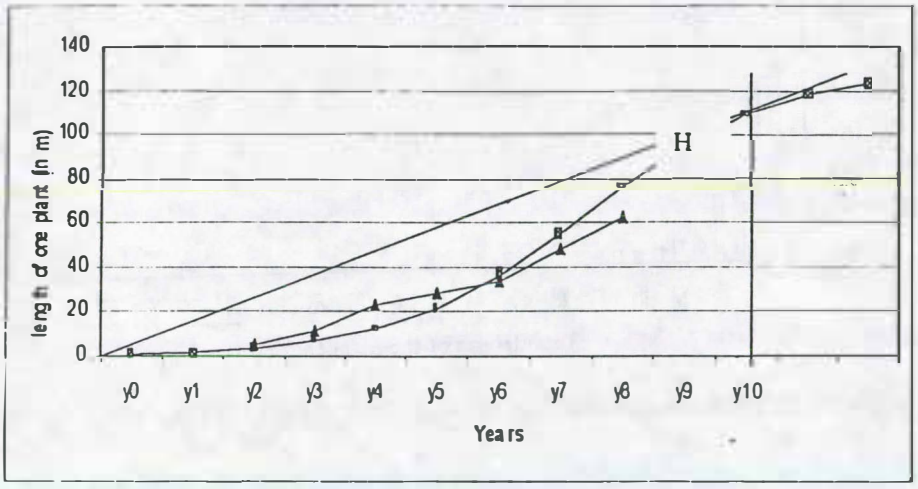
Former assessments of the plots occurred in 1992, 1993, 1994 and 1995. The growth trend may be observed by comparison of the two characters assessed : the number of stems per clump and the length of the stems.

Table 2 : Mean number of stems per clump, according to the age of the plot.

Plot	Year 2	y3	y4	y5	y6	y7	y8
1		3	3	3	4		5
3		2	3	3	4		6
4	2	3	4	5			5
5	5	8	11	16			21
6	6	9	12		19		
8	3	4	6		8		
9	3	3	5		8		
10	3	3	4		7		
11	3	5	8		14		
12	2	3	4		4		
13	2	2	3		3		
14	2	4	6		7		
15	1	2	3		2		
16	4	5	9		13		
18	3	4	5		6		
19	1	1	1		1		
Average	3	4	5	7	7		9
Stdev	1	2	3	6	5		8
CV	49%	56%	57%	92%	71%		85%

and current annual increment will match each other (Point H in Figure 2). The mean total length of one plant will be around 100-110 m.

Figure 2 : Evolution of the mean length of one clump according to its age.



* Δ = Data assessed in the plots ; □ = Estimation with the model.

Estimation of the length of mature cane and the expected income in 1997.

The estimation of the total length of mature cane in 1997 was made following two scenarios. The first one (Table 4) estimates the percentage of mature cane according to the age of the plot : all the canes of one clump were attributed the same age (age of the plot). The mean estimated value of mature cane thus computed was around 45%. The second one (Table 5) estimates the percentage of mature cane according to the estimated mean length of the stem. The mean estimated value was around 30%.

Legend used in Tables 4 and 5.

YOP	* Year of Planting
NbSt97	* Average number of stems per plant
LgPlt	* Average length per plant, estimated from regression made with previous data from 1992 to 1995
Surv	* Number of survival plants per plot
LgTot/ha	* Total length per ha *(=LgPlt x 660 x Alive / 30)*
%MatC	* % of mature cane, estimated from regression from previous yield data (Alloysius 1998) - Data were assessed for stems over 2 meters.
LgMat/ha	* Length of mature cane per ha (calculated for stems over 2 meters only)
Income/ha	* Total price in RM from harvesting per ha

Table 3 : Mean length of one plant according to the age of the plant (in m).

Plot	Year 2	y3	y4	y5	y6	y7	y8
1		5.95	10.40	13.70	18.89		23.62
3		8.34	17.79	27.30	38.51		48.69
4	4.62	11.80	20.69	36.87			45.19
5	14.22	33.84	59.05	104.14			131.78
6	10.20	26.77	53.60		86.10		
8	8.67	15.60	31.26		38.16		
9	3.97	4.69	20.49		33.49		
10	6.97	12.47	22.54		45.60		
11	5.24	12.02	32.89		63.08		
12	5.71	10.05	18.37		23.02		
13	2.15	3.82	7.32		7.72		
14	5.71	9.30	19.80		24.04		
15	1.20	2.29	3.68		3.33		
16	3.98	9.65	27.36		45.60		
18	7.10	13.51	24.77		30.27		
19	0.34	0.74	1.38		1.35		
Average	**5.74**	**11.23**	**23.39**	**45.23**	**33.40**		**62.37**
Stdev	**3.66**	**8.66**	**15.79**	**4.23**	**23.21**		**47.62**
CV	**64%**	**77%**	**67%**	**9%**	**70%**		**76%**

Growth Model

Figure 2 shows the evolution of the mean length of one clump according to its age. A logistic model of growth was estimated according to three parameters : the length of the plant at planting (xo), the growth rate (r), and the potential maximum height of the plant (K). The model gives the length of the plant according to its age (y)

$$Length\ (y) = \frac{K.xo * 2.718^{r.y}}{K - xo + xo * 2.718^{r.y}}$$

At planting, the plants were already almost 1 meter long. At year 8, the maximum length observed in the plots was 137 m, and we may assume that plants may have a maximum length of 130 m in average conditions. We then computed our model using the following values for the K and xo :

- K = 130 m.
- xo = 1 m.

The growth rate was adapted to fit the actual data (r = 0.65). Figure 2 shows the actual data compared to the computed model. With satisfactory conditions of growth, we may conclude that harvesting should occur at 10 years old, when the mean annual increment

Table 4 : Scenario 1 - Estimation of the length of mature cane per plot, and the expected income in 1997.

Plot	YOP	Age	NbSt97	LgPlt #	Surv	LgTot /ha	Scenario 1 % MatC	LgMat /ha	Income/ha
1	Apr-89	8.3	5	23.62	29	15070	55%	7586	531
3	Apr-89	8.3	6	48.69	26	27849	55%	14688	1028
4	Jun-90	7.1	5	45.19	23	22864	48%	10489	734
5	Jun-90	7.1	21	131.78	27	78280	48%	37004	2590
6	Jun-90	7.1	19	86.10	21	39779	48%	18651	1306
8	Jan-91	6.5	8	38.16	19	15950	44%	6650	466
9	Apr-91	6.3	8	33.49	28	20629	42%	8147	570
10	Apr-91	6.3	7	45.60	30	30094	42%	12085	846
11	Apr-91	6.3	14	63.08	29	40244	42%	16367	1146
12	Jan-91	6.5	4	23.02	21	10634	44%	4272	299
13	Mar-91	6.3	3	7.72	23	3905	42%	1215	85
14	Mar-91	6.3	7	24.04	28	14810	42%	5703	399
15	Mar-91	6.3	2	3.33	24	1756	42%	294	21
16	Mar-91	6.3	13	45.60	27	27089	42%	10878	761
18	Mar-91	6.3	6	30.27	23	15319	42%	6009	421
19	Apr-91	6.3	1	1.35	19	563	42%	237	17
			AVG	40.69	25	22802	45%	10017	701

All lengths are given in meters

Table 5 : Scenario 2 - Estimation of the length of mature cane per plot, and the expected income in 1997.

Plot	YOP	Age	NbSt97	LgPlt #	Surv	LgTot /ha	Scenario 2 % MatC	LgMat /ha	Income /ha
1	Apr-89	8.3	5	23.62	29	15070	31%	4276	299
3	Apr-89	8.3	6	48.69	26	27849	31%	8279	580
4	Jun-90	7.1	5	45.19	23	22864	30%	6556	459
5	Jun-90	7.1	21	131.78	27	78280	30%	23128	1619
6	Jun-90	7.1	19	86.10	21	39779	30%	11657	816
8	Jan-91	6.5	8	38.16	19	15950	29%	4383	307
9	Apr-91	6.3	8	33.49	28	20629	28%	5431	380
10	Apr-91	6.3	7	45.60	30	30094	28%	8057	564
11	Apr-91	6.3	14	63.08	29	40244	28%	10911	764
12	Jan-91	6.5	4	23.02	21	10634	29%	2816	197
13	Mar-91	6.3	3	7.72	23	3905	28%	810	57
14	Mar-91	6.3	7	24.04	28	14810	28%	3802	266
15	Mar-91	6.3	2	3.33	24	1756	28%	196	14
16	Mar-91	6.3	13	45.60	27	27089	28%	7252	508
18	Mar-91	6.3	6	30.27	23	15319	28%	4006	280
19	Apr-91	6.3	1	1.35	19	563	28%	158	11
			AVG	40.69	25	22802	29%	6357	445

All lengths are given in meters

Table 7 : Total length of mature cane and expected income at harvesting time (10 years).

Plot	YOP	LgTot/ha (in m)	% MatC	LgMat/ha (in m)	Income/ha (RM)
1	Apr-89	28072	20.5%	5764	403
3	Apr-89	48620	29.3%	14227	996
4	Jun-90	51106	35.8%	18278	1279
5	Jun-90	78408	14.4%	11264	788
6	Jun-90	57750	15.2%	8779	615
8	Jan-91	46398	28.9%	13400	938
9	Apr-91	65912	28.2%	18592	1301
10	Apr-91	77220	32.3%	24936	1746
11	Apr-91	79750	20.8%	16587	1161
12	Jan-91	40656	37.3%	15176	1062
13	Mar-91	13662	20.9%	2862	200
14	Mar-91	54208	27.1%	14675	1027
15	Mar-91	4224	6.1%	257	18
16	Mar-91	69498	20.9%	14556	1019
18	Mar-91	52118	32.8%	17085	1196
19	Apr-91	836	0.0%	0	0
	AVG	48027	23.2%	12277	859

Discussion

The general results of growth recorded in 1997 showed a high variability. Some clumps had already reached a total length over 100 meters and developed several stems, while at the same time some were still monostem plants less than 1 meter long. This great heterogeneity can not be attributed to the environmental conditions only, considering the small area of the plots. More probably some strong and influent genetic factors or or developmental obstacle since the nursery play a key role in the rattan growth.

The comparison between the actual data in 1997 and the model of growth shows that there was a first stage of rapid growth. This growth may be the consequence of the manipulation of the canopy by removal of the trees along the lines that allowed more light to reach the young plants. Then, the canopy covered the opening, and the plants slowed down their growth rate. Kotulai (1998) also observed that in some plots of LFC's *C. caesius* plantations, up to 95% of the plants had at least 2 stems infested by pests (mainly beetles) The stems, infected mainly at the shoot tips, die or have a slower growth. We may then expected the slowdown to continue, and the model will therefore overestimate the yield for the next few years. However, we may conclude that under normal conditions, harvesting should occur at 10 years old, when the mean annual increment and current annual increment will match each other (Point H in Figure 2). The mean total length of one plant will be around 100-110 m.

Prediction of the length of mature cane and the expected income at harvesting (10 years old).

Based on the data recorded by Alloysius (1998), we calculated the percentage of mature cane according to the length of the stem. The relation is given by the logarithmic formula below :

$$\%mat = 0.1833 * \ln(L) - 0.1933$$

where L is the length of the stem in meters.

The correlation between the two characters is very high (R^2=0.72) and thus may be considered as a good mean to estimate the length of mature cane for one stem, regardless of its age. However, since the only character assessed in 1997 was the number of stems, the model can not be applied to the individual length of each stem. We therefore applied the model to the mean calculated length of the stem for one clump.

We previously assumed that harvesting should occur at age 10 years old, according to the model of growth. The same model was used to predict the mean total length of the plants per plot at age 10 years. K and xo were unchanged, but r was modified in order for the total length in 1997 to be included into the model. With the 1997 assessment, we found the following values for r and the corresponding mean total length for the plants, as given in Table 6.

Table 6 : Values of r per plot that better fit the model according to the actual total length in 1997, and average total length of the plant estimated for age 10 years (in m).

Plot	r	Avg Tot Lgth	Plot	R	Avg Tot Lgth
1	0.42	44	11	0.81	125
3	0.55	85	12	0.56	88
4	0.61	101	13	0.35	27
5*	-	137	14	0.56	88
6	0.80	125	15	0.21	8
8	0.66	111	16	0.71	117
9	0.64	107	18	0.62	103
10	0.71	117	19	0.05	2

* The total length in 1997 is already above the model limit K.

Based on the average total length, we computed the average length of one stem, assuming the hypothesis that only the stems present in 1997 will be mature in 2 to 3 years, and that no more mortality will be recorded during the last 2 to 4 years. Then, following the same process as in Table 5, we calculated the length of mature cane and the expected income per hectare (Table 7).

The two scenarios for the estimation of mature length in 1997 may both be considered too optimistic, since in this model we assumed that the percentage of mature cane was linked to the length of the stem by a linear function. It was found later that it is closer to a logarithmic function. The first scenario also estimates the length of mature cane based on the age of the plot. Since *C. caesius* is a multistem plant, younger stems developed at later stages were also considered as old as the first stem, though younger, and therefore less mature.

The prospects for harvesting at 10 years should be closer to the reality because in this case we used the logaritmic model. However, this figure still gives a low expected income unable to cover the planting and maintenance costs (around 2,000 RM/ha) and the harvesting cost (around 3,000 RM/ha).

Conclusion

Planting of *C. caesius* in Luasong Forestry Centre area proved to be non-viable with the present methods and conditions, and the present market price. Moreover, during the planting exercise, site-matching was not given sufficient attention, as well as plot maintenance, and subsequently the growth was not as satisfactory as expected. We might achieve better results with a better choice of the plots to be planted, and with improved genetic material.

Nevertheless, the small size of the yield plots may not enable a good estimation of the potential results in real plantation conditions. The results of the harvesting of the Plantation Unit's plots will probably bring along new enlightenment's about the profitability and viability of rattan plantation in LFC.

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Appendix 7

List of tree trial plots

Species	Trial	Trial no.	Location/Plot	Area (ha)	Date plant
Tectona grandis	Provenance/progeny	TGR1	Taliwas / KM18	5	Mar-97
Tectona grandis	Provenance/progeny	TGR2	LFC/Compt. 311C	4.5	May-97
Tectona grandis	CCT	TGR3	LFC/Compt. 311B&D	7	Aug-Nov 1
Tectona grandis	Comparison of Propagation method	TGR4	Taliwas / KM 18	2.2	Sep-98
Acacia mangium (PNG)	S. Stand/Prov./Prog.	SSO	LFC / Tiagau	3	Feb-90
A. mangium (QLD)	S. Stand/Prov./Prog.	SSO	LFC / Tiagau	3	Apr-90
A. mangium (PNG)	S. Stand/Prov./Prog.	PNG4	LFC / Tiagau	1	Jul-97
A. crassicarpa	S. Stand/Prov./Prog.	SSO	LFC / Tiagau	5	Apr-90
A. auriculiformis	S. Stand/Prov./Prog.	SSO	LFC / Tiagau	3	Apr-90
A. aulococarpa	S. Stand/Prov./Prog.	SSO	LFC / Tiagau	3	Nov-91
Octomeles sumatrana	Thinning		Taliwas / KM 13	5	Apr-96
Gmelina arborea	Progeny trial	GAR1, GAR2	LFC / Tiagau	5	Apr-91
Khaya ivorensis	Provenance (Open)	KIV1	LFC / Tiagau	0.5	Sep-90
K. ivorensis	Progeny (Line)	KIV3	LFC / Tiagau	1.2	Jun-91
K. ivorensis	Progeny (Line)	KIV4	LFC / Tiagau	0.5	Jul-91
Xylia xylocarpa	Species trial (Open)	XXY1	LFC / Tiagau	0.4	Sep-90
X. xylocarpa	Species trial (Line)	XXY2	LFC / Tiagau	0.3	Sep-90
Eucalyptus pellita	Seed Stand	EUSO	LFC / Tiagau	8	Nov-92
Eucalyptus pellita	Clonal Trial	EPCT1	Taliwas / KM18	0.2	Dec-98
Eucalyptus pellita	Clonal Trial	EPCT2	LFC / Comp. 311	0.3	Feb-99
			TOTAL	58.1	

Appendix 8

Average values for several characters of the hybrid clones in the Brumas trial AH18c, ranked by volume. Only the clones with more than 50% survival are represented. The trial is a single-tree RCB with six repetitions.

Clone number	Maternal tree number	Number of living trees	Diameter	Height	Straightness	Fork	Fork number	Branch persistance	Branch size	Branch angle	Volume
116	AA7f	3	23.03	18.86	3.28	3.03	2.71	2.76	2.70	3.35	0.39
72	AA7f	4	21.39	19.10	3.02	2.41	1.70	2.69	2.50	2.70	0.34
37	AA7d	3	20.80	19.04	3.04	2.25	1.63	3.64	2.59	2.34	0.32
46	AA7f	3	20.36	18.86	2.95	1.03	1.04	3.76	3.04	3.01	0.31
51	AA7f	4	20.68	17.73	2.96	2.29	1.49	3.02	2.77	3.21	0.30
79	AA7f	3	20.54	17.72	3.63	2.14	2.08	2.72	2.71	2.67	0.29
134	AA7f	3	19.93	18.69	3.28	1.36	1.37	2.76	2.70	3.01	0.29
41	AA7d	6	20.38	17.83	3.33	2.50	2.33	3.00	3.00	3.00	0.29
18	AA7d	4	20.18	17.73	3.21	2.04	1.49	3.27	2.77	2.71	0.28
107	AA7f	3	20.31	16.72	3.30	2.81	2.41	3.72	2.71	2.67	0.27
29	AA7d	3	19.85	17.25	3.04	1.97	2.00	2.95	3.04	3.40	0.27
118	AA7f	3	19.74	16.48	3.72	2.97	2.29	3.24	2.96	3.65	0.25
13	AM7c	3	18.99	17.21	2.96	1.09	1.02	3.08	3.28	3.36	0.24
24	AA7d	3	18.73	17.02	3.28	2.36	1.71	2.76	2.70	3.35	0.23
140	AA7h	3	18.28	17.55	3.30	1.14	1.08	3.05	2.38	2.00	0.23
7	AM7c	3	18.73	16.48	3.37	2.25	2.33	3.68	2.66	3.42	0.23
2	AM7b	3	17.80	17.71	3.37	2.92	1.96	3.64	3.25	3.67	0.22
16	AA7d	3	18.06	16.88	3.29	2.75	1.68	3.74	2.62	3.36	0.22
14	AA7d	4	17.44	17.68	2.96	2.09	1.52	3.04	3.24	3.72	0.21
144	AA7h	3	16.97	18.14	3.05	0.97	0.96	2.58	2.96	3.32	0.21
84	AA7f	4	17.59	16.57	3.02	1.96	1.71	2.72	2.98	2.77	0.20
49	AA7f	4	17.44	16.50	3.46	2.59	2.05	3.32	3.30	3.03	0.20
43	AA7f	3	17.72	15.75	2.96	3.03	2.00	3.71	2.63	3.26	0.19
80	AA7f	3	16.71	17.55	3.38	1.31	1.36	2.61	2.35	2.98	0.19
40	AA7d	3	17.07	16.48	3.38	2.64	1.63	2.91	2.96	2.99	0.19
57	AA7f	3	17.18	16.25	3.71	2.97	2.66	3.62	2.70	2.74	0.19
99	AA7f	3	17.18	16.18	3.38	3.03	2.37	2.64	3.00	3.08	0.19
127	AA7f	4	17.17	16.20	3.52	1.71	1.46	3.22	2.48	2.77	0.19
78	AA7f	5	16.72	16.89	3.61	1.78	1.39	3.21	3.18	2.84	0.19
62	AA7f	3	17.07	15.79	3.63	1.08	1.04	3.03	3.75	3.00	0.18
48	AA7f	4	16.45	16.70	3.27	2.50	1.76	2.70	3.06	3.75	0.18
130	AA7f	3	16.40	16.18	3.38	1.91	1.28	2.55	2.31	2.23	0.17
50	AA7f	3	16.27	16.29	3.63	2.08	1.70	3.36	2.41	2.66	0.17
88	AA7f	3	17.15	14.65	3.62	1.97	1.96	3.02	2.33	2.59	0.17
131	AA7f	5	17.01	14.27	3.41	2.18	1.58	3.56	2.81	3.37	0.16
94	AA7f	3	15.75	16.51	3.72	1.03	1.04	3.31	3.00	3.08	0.16
137	AA7h	4	15.55	16.85	3.52	3.00	2.27	3.77	3.26	3.33	0.16
60	AA7f	5	15.79	16.07	3.21	2.58	1.78	2.76	2.81	2.77	0.16
95	AA7f	4	16.11	15.28	3.03	1.54	1.54	2.97	3.03	3.26	0.16
128	AA7f	3	16.01	15.35	2.94	0.97	0.98	2.78	2.94	3.37	0.15
9	AM7c	3	15.68	15.22	2.97	1.81	1.41	3.05	3.04	3.34	0.15
30	AA7d	4	16.15	14.23	3.45	1.00	1.00	3.32	3.49	3.48	0.15
97	AA7f	4	15.08	16.25	4.01	2.67	1.72	2.76	3.20	3.03	0.15

Ranking, for several characters of the hybrid clones in the Brumas trial AH18c. Only the clones with more than 50% survival are represented.

Clone number	Maternal tree number	Number of living trees	Diameter	Height	Straightness	Fork	Forking number	Branch persistance	Branch size	Branch angle	Vol
89	AA7f	3	15.37	15.62	2.96	1.75	1.37	3.03	3.08	3.66	0.
122	AA7f	3	15.39	15.55	2.96	2.09	1.35	2.08	2.95	2.69	0.
8	AM7c	5	15.24	15.19	3.41	2.18	1.59	3.21	2.98	2.64	0.
87	AA7f	3	15.24	14.68	3.05	1.25	1.28	3.21	2.31	2.23	0.
135	AA7f	3	15.12	14.68	3.05	1.37	1.37	3.64	3.00	3.41	0.
125	AA7f	3	15.77	13.35	3.61	1.97	1.98	4.12	2.94	3.37	0.
56	AA7f	3	15.04	14.55	3.63	2.14	1.41	3.39	3.04	2.67	0.
4	AM7b	4	14.44	15.52	3.52	1.75	1.49	2.48	2.96	3.27	0.
34	AA7d	3	14.86	14.65	3.29	2.09	1.72	3.12	3.02	3.44	0.
138	AA7h	4	14.80	14.75	3.53	1.50	1.23	2.66	3.00	3.19	0.
20	AA7d	4	14.39	15.18	3.02	1.71	1.48	2.46	2.47	2.70	0.
115	AA7f	4	14.40	14.62	3.20	1.79	1.76	3.09	3.22	3.05	0.
64	AA7f	4	14.07	15.30	3.77	2.21	1.48	2.96	2.47	2.45	0.
68	AA7f	5	15.15	13.09	3.81	2.22	1.63	3.40	3.03	3.03	0.
86	AA7f	4	14.35	14.28	3.45	1.75	1.50	3.07	2.74	2.48	0.
121	AA7f	4	13.77	14.63	3.21	1.34	1.30	2.57	2.55	2.78	0.
65	AA7f	4	13.70	14.50	3.53	1.25	1.23	2.91	3.25	3.19	0.
136	AA7f	4	13.59	14.35	3.77	1.41	1.20	3.44	3.00	3.45	0.
142	AA7h	3	14.63	11.69	3.28	2.03	1.37	3.09	3.04	2.68	0.
19	AA7d	3	13.39	13.59	3.29	1.03	1.00	3.38	2.96	3.60	0.
25	AA7d	3	12.70	15.04	3.04	1.92	1.30	3.64	2.59	3.00	0.
76	AA7f	3	13.28	13.72	3.38	1.97	1.36	3.95	3.02	3.65	0.
67	AA7f	3	12.94	13.96	3.63	1.08	1.04	3.36	2.75	3.66	0.
36	AA7d	4	12.87	13.32	3.27	1.21	1.21	3.22	2.73	3.27	0.
33	AA7d	3	12.93	12.98	3.37	1.58	1.33	2.68	2.66	3.09	0.
105	AA7f	3	12.96	12.88	3.37	0.92	0.96	2.31	2.59	3.34	0.
133	AA7f	4	12.26	14.05	3.46	2.09	1.52	3.54	2.24	2.97	0.
45	AA7f	3	11.57	14.19	2.97	1.08	1.00	3.32	3.01	2.91	0.
141	AA7h	3	11.23	13.98	3.29	2.09	1.39	2.79	2.69	3.77	0.
31	AA7d	4	12.19	11.80	3.46	2.84	2.27	3.29	3.24	3.22	0.
129	AA7f	3	10.71	13.81	4.05	1.64	1.29	3.58	3.30	3.32	0
117	AA7f	3	10.95	10.95	2.95	1.69	1.31	3.39	2.98	2.35	0
54	AA7f	3	9.17	10.52	3.63	1.08	1.00	2.32	2.34	2.58	0.

Note: Discrete characters scores are as follows:

Straightness score: from 1 (best) to 6 (worst)

Fork score: 1=no fork, 2=upper third of the tree; 3=middle of the tree; 4=lower third of the tree

Forking number: counts the number of forks in a tree, 0=no fork, 1=one fork, up to 4.

Branch persistance score: from 1 (good self-prunning ability) to 6 (poor ability)

Branch size score: 1=small diameter branches; up to 5=large diameter branches

Branch angle score: 1=horizontal, 5=parallel to the tree

Maternal tree: AA= Acacia auricoloform
AM= Acacia mangiun

Date plantation: September 199

Date last assessment: August 1998

Appendix 10

Acacia mangium Clone no. 5. Comparison of *in vitro* vs seedlings.

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Introduction

This experiment was established with the main objective of evaluating two propagation methods (tissue culture and seeds) for *Acacia mangium*. The hypothesis to test is that clonal propagation of superior trees gives more homogeneous and better performing material than seeds obtained by the same trees, which contain an unknown male contribution. A first assessment made in March 1998 (15 months after planting) yet yielded no difference between *in vitro* and open-pollinated trees. Results of the third assessment made in February 1999 are given in this paper.

Material and method.

The trial was planted in Nov 1, 1996, using both the *in vitro* plantlets (treatment 1, T1) and open pollinated seedlings from clone n. 5 (treatment 2, T2). The two treatments were planted in a Random Complete Block design with three repetitions (R1, R2, R3), at a spacing of 3 x 3 meters. Around the trial, a two-line buffer (B) was planted with seedlings obtained from a non selected seed bulk.

Data recorded during the assessment of February 1999 were the diameter at breast height (DBH, in cm), the height (in cm), the straightness and the branching / forking of the trees. Continuous variables (DBH, height) were analysed using a classic ANOVA with block, treatment and their interaction as independent factors; block and treatment effect were tested against their interaction. Non-parametric variables (forking, straightness) were tested using non-parametric tests (SAS [1988] NPAR1WAY procedure: Wilcoxon 2-sample test, T-Test and Kruskal-Wallis Test). To test the hypothesis that clonal material is more homogeneous than seedlings, the height and DBH of the two treatments were compared with the Bartlett's test evaluating the difference between variances (Sokal & Rohlf, 1981).

Results

The analysis of the 1999 assessment showed the same pattern observed previously in the 1998 assessment, i.e. that 27 months after planting there were no significant differences between treatments or blocks neither for diameter nor for height (Table 1) The

significant interaction (repetition*treatment) was just due to the fact that one treatment (T1) was superior to the other (T2) in one repetition (R2) but not in the other two (R1 and R3; not shown); however, most probably this was due to the low number of trees within the experimental unit rather than to a real interaction.

Table 1. Analysis of variance for continuous characters

HEIGHT

Source	Degrees of freedom	Sum of squares	F-test Value	Prob > F
BLOCK	2	50565.77	0.43	0.6984
TREAT	1	112804.30	1.93	0.2995
BLOCK*TREAT	2	117087.86	3.68	0.0324

DBH

Source	Degrees of freedom	Sum of squares	F-test Value	Prob > F
BLOCK	2	50565.77	0.43	0.6984
BLOCK	2	2.13	0.23	0.7963
TREAT	1	6.73	1.44	0.2352
BLOCK * TREAT	2	13.91	1.49	0.2350

The ranking of the treatments showed a slightly better performances of seeds as compared to micro-cuttings, however the Duncan's test revealed (in concordance with the analysis of variance) that the differences in ranking was not significant (Table 2).

Table 2. Avera e hei ht (in m) and DBH (in cm) er block and er treatment.

	Treatment	Block 1	Block 2	Block 3	Avera e
Height	Micro cuttings	13.67	12.40	11.91	12.72
	Seedlings	13.28	13.92	13.52	13.57
	Average	13.46	13.20	12.86	
	Treatment	*Block 1*	*Block 2*	*Block 3*	*Avera e*
DBH	Micro cuttings	12.10	11.64	10.53	11.50
		11.55	12.35	12.49	12.13
	Average	11.81	12.02	11.68	11.89

Figure 1 shows again the comparison of the two treatments. In spite of the fact that no significant differences could be detected, seedlings appear to be superior in term of growth as compared to *in vitro* cuttings. The failure of the experimental design and the statistical analysis to capture a difference may be due to the small number of trees in the trial.

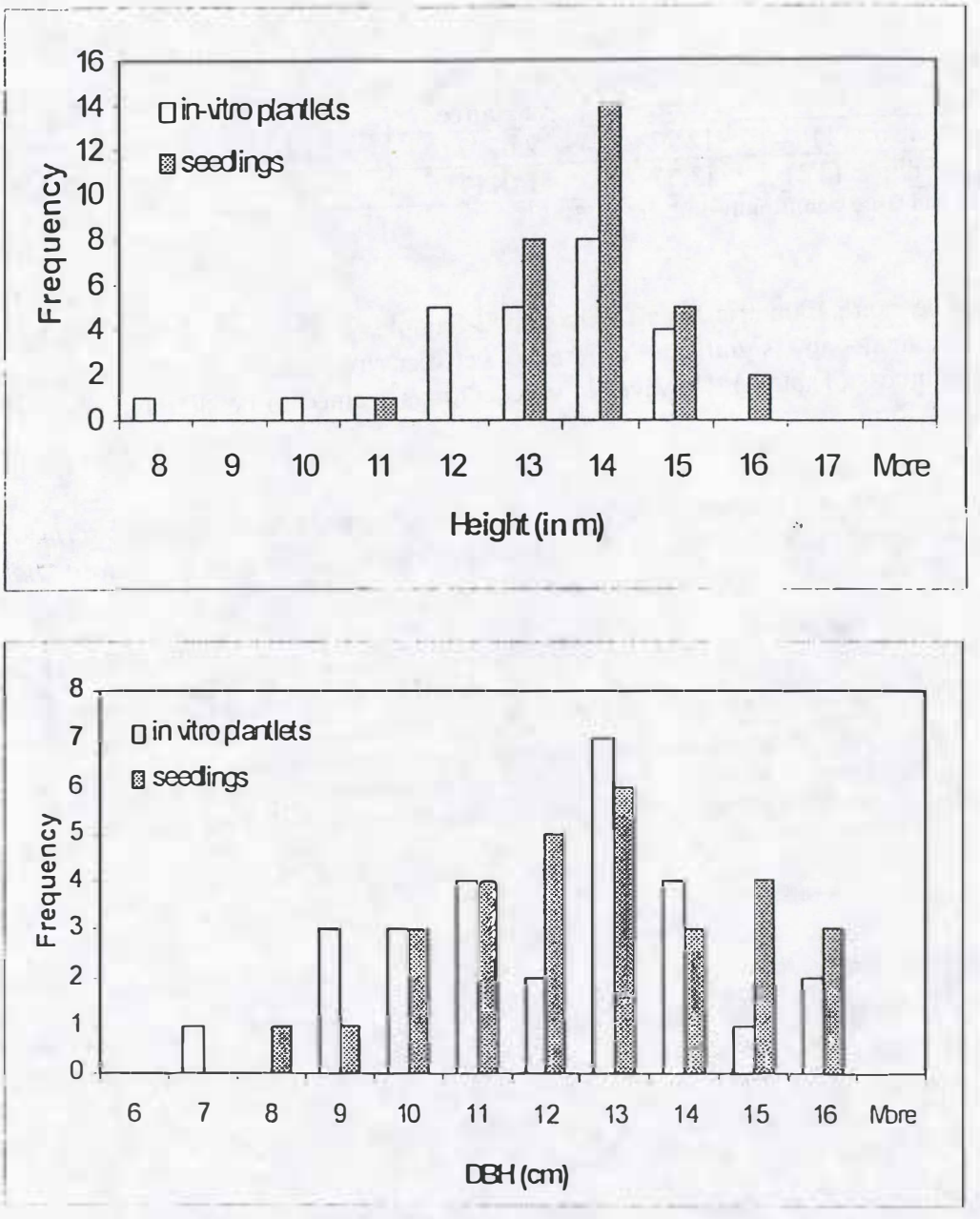

Figure 1. Total frequency distribution of height and diameter according to the treatment (propagation method) in the trial.

The test of Bartlett did not highlight any significant difference between the variance (the two treatments for the DBH (P=0.46). In contrast, a significant difference in varian

appeared for the height (P=0.014), treatment 2 (seedlings) being more homogeneous than treatment 1 (*in vitro* plantlets; Table 3).

Table 3. Dispersion parameters for the height (meters)

	Average	Variance	SD	CV*
In vitro (T1)	12.72	2.6767	1.64	13% A
Seedlings (T2)	13.57	1.0117	0.73	7% B

* A and B are significantly different at 5%.

The non-parametric tests (Wilcoxon 2-Sample, T-Test and Kruskal-Wallis) did not discriminate any significant difference between treatments for neither branching nor straightness (Table 4). However in vitro cuttings seemed to be sligthly better in terms of trunck form.

Table 4. Comparison of seedlings and cuttings in term of trunck form. A lower score indicates less trunck defects. The differences are not significant at the statistical test.

Treatment	Branching	Straightness
Seedlings	0.23 (ns)	0.20 (ns)
In vitro cuttings	0.16 (ns)	0.12 (ns)

Note: Code for branching: 0=no branching, 1=aerial branching, 2=branching from the bottom or forking; Code for straightness: 0=straight, 1=slightly crooked, 2=very crooked. (ns)=not significant different from the other treatment.

Conclusion

At 27 months after plantation, the only significant difference between micro-cuttings and seedlings of *A. mangium* clone n. 5 was found for the homogeneity of height growth, the seedlings being less variable than cuttings. This finding may point to problems inherent to the micro cutting technique adopted for this experiment, including the nursery operations following the acclimatisation.

No other significant differences could be detected neither in growth or in trunck form between seedlings and micro cuttings. Interpreting this negative result requires noting that the trial was small in size, including only 30 trees per treatment, and only one maternal genotype was represented (clone n. 5)

However, seedlings appeared to be slightly superior to micro cuttings in terms of average growth. Possible explanations for this are: 1) Clone n. 5 is not superior to the average paternal tree of the seedlings; in other words, it has no genetic advantages

compared to the average tree in the plot. It has to be noted that the trial has been selective thinned twice before the material was collected, so that the worst genotypes were discarded and could not contribute to the progeny. 2) again, problems of the micro propagation technique could be responsible for the slower growth.

The other possible explanation involving maternal effects, which has been invoked earlier (Bacilieri *et al.*, 1998), may be by now discarded discarded considering the age of the trial that should have erased any of these effects.

A larger experiment with more plants per treatment and more maternal genotypes will help to better evaluate the genotype and environment effect on *A. mangium* growth.

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Appendix 11

A. mangium x A. auriculiformis hybrids. Rooting of cuttings from 1.5-year old trees.

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Introduction

To date, little information is available on the rooting capacity of Acacia hybrid in the nursery, while that of *A. mangium* has been widely studied. Kendel (1987) reported the inability of *A. mangium* cuttings to root without hormone, while a percentage of 51% was obtained with hormone treatment (IBA, NAA[1], or both). A node effect was also recorded - the terminal cuttings being easier rooted than those of inferior ranks. For hybrids, Wongmanee (1989, in Kijkar, 1992) proved the feasibility of propagation with cuttings, at least when cuttings were issued from hedged and rejuvenated plus tree. In order to see whether propagation by cutting may be successful with cuttings from non-felled trees, we started an experiment on *A. mangium x A. auriculiformis* hybrids aimed at collecting data on their rooting ability.

Material and method.

The material used for this experiment was collected on 8 hybrids brought back from Ivory Coast and planted in the LFC[2] in 1997 (AH8 to AH17), next to a small plot of *A. mangium* clone 5, out of which 2 (AM1 and AM2) are used here as a reference. Shoots were collected in the upper part of trees after they were felled using chainsaw for the coppicing experiment. Four shoots were selected and cut into 8 single-node cuttings. For clone AH8 and AH10, we alternated one simple-node cutting with one double-node cutting. On some trees signs of bark and leaf disease were observed. Two of the trees had quite round leaves, closer in shape to that of *A. mangium* than to hybrids.

The eight cuttings were disposed on the bed in decreasing order to trace them until the end of the experiment, cutting n. 1 being the one at the top, and n. 8 at the bottom of the shoot. Besides the 4 shoots, for each tree 4 cuttings from 2 axiliary shoots were added, in the same way: oriented from the top to the base (A1 to A4).

The leaves were trimmed at a third or fourth of their length. The cuttings were then bathed in a Thiram solution, then half of them were dipped into rooting hormone powder

[1] IBA = Indol butyric acid ; NAA = Naphtalen acetic acid.
[2] Luasong Forestry Centre, Sabah.

(Seradix 3) at the base of the cuttings (Figure 1), while the after half was introduced in the mist beds without hormone treatment.

Figure 1: individual layout for cuttings of one tree in the mist chamber.

Seradix 3	Ax. shoot			⊕	⊕	⊕	⊕		
	Main shoots	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
		⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
No Seradix 3	Main shoots	O	O	O	O	O	O	O	O
		O	O	O	O	O	O	O	O
	Ax. shoot			O	O	O	O		

Basal → Top

Introduction of the explants into the mist nursery occurred just after collection from the trees, on November 12, 1998. A mortality assessment was made 3 weeks after the transfer. The first assessment of rooting (and the subsequent transfer of rooted cuttings to polybags) occurred one month after the beginning of the experiment. Both the number of roots and the length of the longest root (called root1) were recorded. The unrooted cuttings were checked again one week after the first assessment. At this second stage, all the unrooted cuttings were considered as dead.

The rooted cuttings stayed one week under spraying every 3 minutes, then 2 more weeks on a bed sprayed every 10 minutes. They were finally transferred to the greenhouse.

Results and analysis

Mortality assessment

The results concerning mortality at three weeks after introduction of the explants in the mist (Tables 1 and 2) were analysed by a Khi2 formula given by Brandt and Snedecor (cited by Cochran & Cox, 1957, Experimental Designs, second edition. p103):

$$\chi 2 = [\Sigma (di.pi) - \rho \Sigma (di)] / \rho.q$$

di being the number of dead under the ith treatment,
pi the percentage of dead under the ith treatment,
ρ the average percentage of dead,
$q = 1 - \rho$.

Table 1 : Observed number of dead cuttings for the lots without hormone three weeks after introduction.

NoH	AM1	AM2	AH8	AH10	AH11	AH12	AH14	AH15	AH16	AH17
Dead	13	13	7	3	10	2	7	7	1	3
Survival	7	7	13	17	10	18	13	13	19	17
Total	20	20	20	20	20	20	20	20	20	20
% dead	0.65	0.65	0.35	0.15	0.50	0.10	0.35	0.35	0.05	0.15

Table 2 : Observed number of dead cuttings for the lots treated with Seradix 3 three weeks after introduction.

H+	AM1	AM2	AH8	AH10	AH11	AH12	AH14	AH15	AH16	AH17
Dead	7	10	8	9	9	2	0	3	0	4
Survival	13	10	12	11	11	18	20	17	20	16
Total	20	20	20	20	20	20	20	20	20	20
% dead	0.35	0.50	0.40	0.45	0.45	0.10	0.00	0.15	0.00	0.2

The value calculated for Khi2 for the whole experiment (Table 1 and 2) was 75.9 (d.f=19) which corresponds to a probability of less than 0.001. Therefore the differences between lots (clones and species) were highly significant. The analysis was pursued using 2x2 contingency tables for Khi2 analysis, to highlight which factors influenced the differences. It was found that :

- The difference between *A. mangium* and hybrids was significant at the 0.1% level
- The difference between treatments (hormone/no hormone) was not significant.

Acacia mangium suffered comparatively a higher mortality than the hybrids. On the other hand, treatment with rooting hormone seemed not to have any significant effect on the survival rate after 3 weeks.

Assessment of rooting

Results of the assessment of rooting rates are given in Table 3, while Table 4 and 5 give the data according to the cutting order. *A. mangium* performed very poorly in this experiment, with only 2 rooted cuttings out of 80. The hormone did not improve the rooting rate.

Table 3 : Rooting rate, average number of roots and average length of the first root, per species . clone, per treatment (Seradix No Seradix) after 4 weeks.

Species	No Seradix 3			Seradix 3		
	Nb of rooted cuttings - %	Avge nb of roots	Avge lgth of root l	Nb of rooted cuttings - %	Avge nb of roots	Avge lgth of root l
AM1	0 – 0%	0.00	0.00	0 – 0%	0.00	0.00
AM2	0 – 0%	0.00	0.00	2 – 10%	2.00	5.25
AH8	0 – 0%	0.00	0.00	7 – 35%	6.28	5.21
AH10	13 – 65%	1.69	7.04	9 – 45%	4.33	5.39
AH11	3 – 15%	1.33	5.17	7 – 35%	4.14	6.50
AH12	12 – 60%	1.33	7.67	13 – 65%	5.92	8.15
AH14	5 – 25%	1.00	5.10	16 – 80%	9.44	7.72
AH15	3 – 15%	1.67	4.00	13 – 65%	6.61	6.08
AH16	5 – 25%	1.20	4.60	18 – 90%	9.94	5.89
AH17	6 – 30%	-	-	15 – 75%	-	-

Figure 2 shows the percentage of rooted cuttings per species / clone, for the two treatments (hormone / no hormone), while Figure 3 and 4 show respectively the average number of roots per cutting, and the average length of the longest root, per species / clone, for both the treatments.

Figure 2 : Percentage of rooted cuttings per species / clone, per treatment.

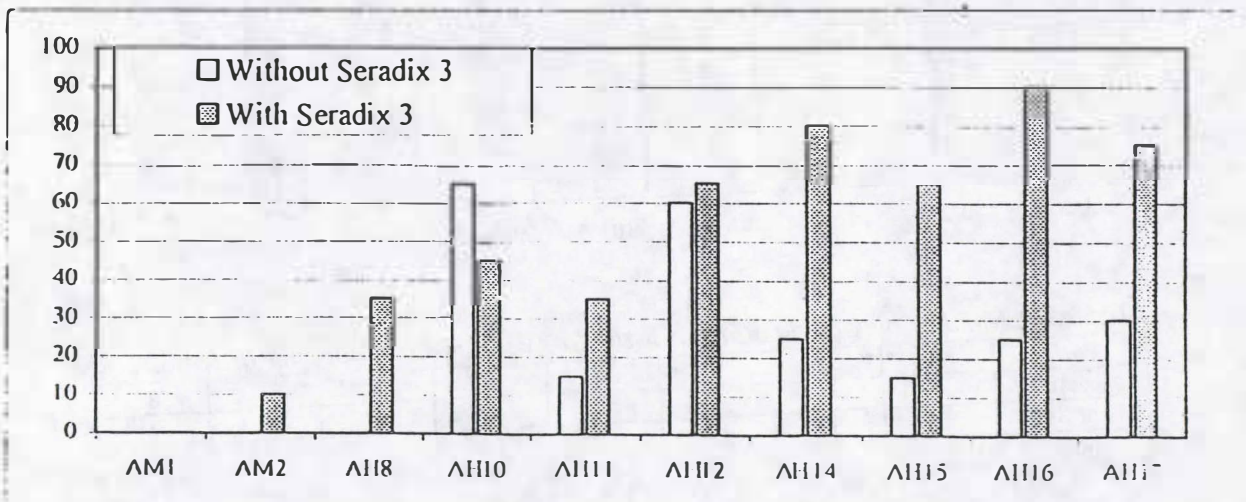

Figure 3 : Average number of roots per rooted cutting per species / clone. per treatment.

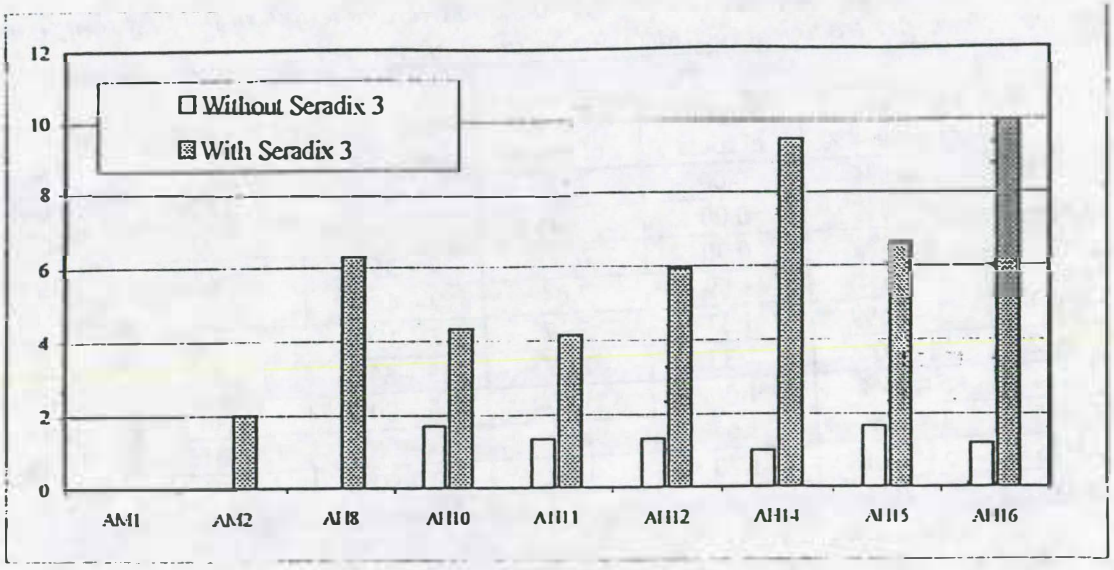

Figure 4 : Average length of the longest root per species / clone. per treatment.

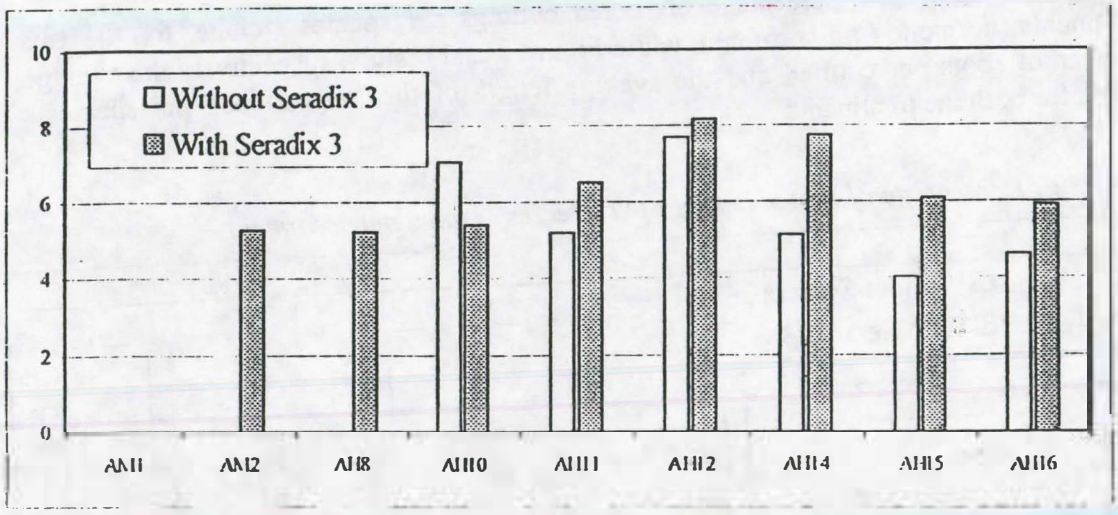

Table 4 : Number of rooted cuttings for the lots without hormone, per order.

NoH	1	2	3	4	5	6	7	8
Rooted	4	9	7	4	6	3	3	2
Non Root	16	11	13	16	14	17	17	18
Total	20	20	20	20	20	20	20	20
% rooted	0.20	0.45	0.35	0.20	0.30	0.15	0.15	0.10

Table 5 : Number of rooted cuttings for the lots treated with Seradix 3, per order.

H+	1	2	3	4	5	6	7	8
Rooted	6	10	16	13	11	9	9	6
Non Root	14	10	4	7	9	11	11	14
Total	20	20	20	20	20	20	20	20
% rooted	0.30	0.50	0.80	0.65	0.55	0.45	0.45	0.30

All these results were analysed using the same method than for mortality. Two by two contingency tables and the Brandt and Snedecor's formula were used for a Khi2 analysis. The results showed that :

- The species / clone present highly significant differences in rooting (P<<0.001), *A. mangium* being far less rooted than hybrids. The following statistical analysis will refer to Acacia hybrids only.
- For Acacia hybrids, the difference between treatment (hormone) is also highly significant at a 0.1 % level (P <<0.001), Seradix 3 favorising the rooting rate. The average rooting rate with hormone was 61% while without hormone was 29%.
- The difference between cutting position on the shoot was also highly significant (P<<0.001).
- There is no difference between the cuttings from the main shoots and those of the axillary shoots (khi2=1 ; 1 d.f).

Final mortality assessment

Finally, a mortality assessment was made 4 weeks after transplantation to polybags. The polybags were then brought to the chamber, under plastic sheet, for further development. The results of the global mortality assessment are given below in table 6.

Table 6 : Survival rate per species, clone and per treatment (Seradix / No Seradix) since the beginning of the experiment, at four weeks after transplanting in the polybags.

Species	No Seradix 3				Seradix 3			
	Total nb of dead cuttings	% of dead*	% of surv 1	% of surv 2**	Total nb of dead cuttings	% of dead	% of surv 1	% of surv 2**
AM1	-	-	0%	0%	-	-	0%	0%
AM2	-	-	0%	0%	20	11%	0%	0%
AH8	-	-	0%	0%	16	23%	57%	20%
AH10	7	0 %	100%	65%	12	9%	89%	40%
AH11	20	25%	0%	0%	15	15%	71%	25%
AH12	11	57%	69%	45%	12	71%	62%	40%
AH14	17	13%	60%	15%	11	75%	56%	45%
AH15	17	0%	100%	15%	11	57%	69%	45%
AH16	17	13%	60%	15%	4	100%	89%	80%
AH17	13	8%	88%	35%	6	20%	93%	70%

Note: * % of dead rooted cuttings. ** % of surv 1 is the survival rate from the transplantation to polybags to the final assessment. % of surv 2 is the survival rate from the beginning to the final assessment.

For the final survival rates, the difference between species / clone (AH or AM), between the cutting orders (1 to 8) and between the treatments (hormone / no hormone) is still highly significant (P <<0.001; this latter being of course determined by the better rooting of hormone-treated cuttings).

Discussion

The first result of this experiment was that of the 80 cuttings of *A. mangium*, only two rooted. This species did not respond to vegetative propagation starting from aged trees. By contrast, another experiment on coppicing showed that young plants produce more responsive material (Schueller *et al.*, this volume).

For Acacia hybrids, the global results can be summarised as follows:

Global rooting rate at transplantation into polybags :	45.3 %
Global survival rate from transplantation to polybags to the final assessment :	74.8 %
Global survival rate from the beginning to the final assessment :	**34.7 %**

Globally, the quite low global rooting-survival rate (35%) was mainly due to the poor rooting rate, which is only 45%. This poor performance can be understood taking into account that the shoots used for this experiment were collected from 1.5-year old trees, and were not juvenile, though taken from the upper part of the tree. Cuttings from juvenile trees should lead to a more successful rate. Experiments to assert this will be carried out soon in LFC, using hybrids young stockplants.

Our experiment highlights that in Acacia hybrids the survival rate during the rooting period (up to three weeks after introduction) is not influenced neither by the clone number, nor by the hormone treatment and the orders of cuttings. The same experiment on *A. mangium* (Kendel, 1987) showed that terminal cuttings had a better survival, 69% of the survival after 9 weeks being from order 1 to 3.

Kendel (1987) using almost the same protocol than us to highlight node, hormone and fungicide effects on *A. mangium* recorded an average rooting rate of 25% after 9 weeks (0% for untreated cuttings, 51% for those with hormone treatment). Our failure to root *A. mangium* can be due to positional effect of the experiment in the nursery (*A. mangium* was by chance near the door and this part of the nursery usually records a lower rooting rate), or to the poor condition of the ramets in the field. By contrast, in our experiment, rooting rate for hybrids was 45% after only 5 weeks.

Wongmanee (cited in Kijkar, 1992) found that for *A. mangium x A. auriculiformis* hybrids, the rooting rate was over 70% (maximum over 90% for two trees) for cuttings collected from shoots sprouting from the lower part of the tree, under 1 meter of height But the rate decreased to 35% between 1 and 3 m, to be reduced to only 7% for shoots sprouting at over 3 meters of height on the trunck. Cuttings from shoots collected in the upper part of the trees therefore have a lower rooting ability. In our experiment, all shoots were collected over 3 meters of height.

Within the Acacia hybrid, hormone was a main factor of rooting, together with the node order. Intermediate-rank cuttings rooted more easily than terminal or basal cuttings On *A. mangium*, Kendel (1987) found no positional effects on rooting ability The hormone was found to increase the rooting ability, while untreated cuttings also rooted, unlike untreated cuttings of *A. mangium*. However, 'axillary' cuttings were found to root with the same ability than those deriving from the main shoots.

Within hybrids, the rooting rates were also significantly different between clones. Some clones performed well irrispectively of the hormone treatment; others rooted poorly without hormone, and their rooting ability increased significantly with the hormone treatment. The hormone treatment also induced highly significant differences for both the number of roots and the length of the longest root. For hybrids propagated under *in vitro* conditions, Galiana (1999) obtained a rooting rate over 70%.

For rooted cuttings, Kendel (1987) recorded an average survival rate of 91% in *A. mangium*, while only 75% of our rooted hybrid cuttings survived. The survival rate of rooted *in vitro* micro-cuttings was also comparatively higher than that of cuttings in our experiment, with a mean of 95.2% (Galiana 1999). The survival rate of unrooted *in vitro* micro-cuttings was also higher, with a mean of 80.5%. We may think that the rooting system develops and perform better for juvenile *in vitro* plantlets than for cuttings. The relatively old age of the collected trees is a factor playing a role in the difference.

Conclusion

Propagation of Acacias hybrids by cuttings from 1.5-year old plus trees proved to be feasible, but a large margin of improvement exists in the technique. In the conditions of our experiment, the age of the trees and their poor sanitary conditions seemed to be the main factors lowering the rooting rate. While rooting mature material looks an interesting perspective to mobilise adult interesting genotypes, more attention should be paid to their sanitary conditions, either using pesticides, fungicides or fertiliser prior to collection.

The survival of rooted cuttings is another factor that can be improved, by a more regular management of the mist system and by a more suitable medium in the polybags. Further experiments on hybrids propagated in the Plant Biotechnology Laboratory and planted in LFC as stockplants, both in polybags and soil, should show whether younger and more responsive material may increase the rooting rate, and thus the survival of cuttings.

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Appendix 12

A. mangium and *A. crassicarpa* Coppicing and Vegetative Propagation Trial.

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Introduction.

In order to gather data about the coppicing and vegetative propagation ability of *Acacia mangium* and *A. crassicarpa*, an experiment was carried out with planting material produced in the PBL[1], and previously brought to the LFC[2] for an acclimatization experiment. The aim of the experiment was to compare the coppicing ability of the two species, and to study the rooting and survival rates of cuttings from clones as compared to bulks. This experiment will also be the prelude to an other experiment made in the same conditions with *A. mangium x A. auriculiformis* hybrids.

Material and method.

The material used for this experiment were clones of *A. mangium* (Am15, Am21 and Am24), a bulk of *A. mangium* from seedlings (Am6) and a bulk of *A. crassicarpa* (AC). Am15 and Am21 were selected in Ivory Coast from mature plus trees, while Am24 originated from non-selected juvenile material from Australia. They have all been maintained under *in vitro* conditions since 1990, acclimatised in January 1998 and transferred to polybags in February 1998.

The plants were hedged the first time at age 8 months. Among the best-looking plants of each lot, we chose 4 to become stockplants for the clones of *A. mangium*, and 8 for each of the two bulks.

The characteristics of the stockplants are given in Table 1. Each stockplant was labeled to enable tracing and comparison of the shoot production along the collections. Cuttings were bulked per clone in the experimental design under the mist.

The first intention was to collect the shoots monthly, but one month after hedging, the shoots were not yet grown enough. We waited the 6th week for the first collection, the second occurred 7 weeks after the first, and the third 6 weeks after the second.

Shoots were collected and their characteristics (stockplant number, length, number of cuttings) recorded. The length was recorded regardless of the presence of leaves on the

[1] Plant Biotechnology Laboratory.
[2] Luasong Forestry Center.

shoot, but the part without leaves was not used for cuttings. For terminal cuttings, we always left the first well-developed leaf, since it was previously observed that cuttings with only the young terminal leaves were doomed. Cuttings were then bathed into a Thiram solution, dipped into a powder rooting hormone (Seradix-3) and put under mist for rooting (10-second burst every 3 minutes).

Table 1 : characteristics of the stockplants.

Species / clone	Stockplant number	Height of the stockplant (cm)	Species / clone	Stockplant number	Height of the stockplant (cm)
AM6	R1	25.0	AM15	R1	17.5
	R2	25.0		R2	19.0
	R3	8.5		R3	19.0
	R4	23.5		R4	13.0
	R5	10.5	AM21	R1	12.5
	R6	25.0		R2	13.0
	R7	22.0		R3	14.0
	R8	19.5		R4	17.5
AC	R1	20 + 17**	AM24	R1	10.0
	R2	20.0		R2	20.0
	R3	25.0		R3	20.0
	R4	21.0		R4	21.5
	R5	20.0			
	R6	16.5			
	R7	20.0			
	R8	16.5			

** Double-stem stockplant ; the shoots were collected regardless of the stem.

The experimental design used was a Random Complete Block design (non equilibrated). We separated the terminal cuttings from the others (intermediates), and made 3 repetitions (blocks) for each. The experimental design and the number of cuttings per species per block is given in Table 2. For the rooting assessment, the presence/absence of roots was recorded (roots were recorded as present when allowing the cuttings to be transferred into polybag). For the survival assessment, the cuttings were recorded as dead or alive. Therefore, the statistic analysis was made for both the variables with a non-parametric test (Kruskal-Wallis non parametric test, with SAS 98).

Table 2 : Number of observations per species/ clone, per block.

Species / clone	Blocks with the terminal cuttings (T1, T2, T3)	Blocks with the remaining cuttings (I, II, III)
AM15	4	15
AM21	4	8
AM24	4	8
AM6	11	24
AC	6	16

To date, three collections were made, but the cuttings of the last one are still in the mist chamber. Stockplants were fertilised after the second and third collection, using NPK. Three holes were dug at 5 cm from the stem, and ten pills put in each hole.

A first assessment for rooting was made 3 weeks after intruduction into the mist, and one week later. At this later stage, unrooted cuttings were considered as dead. Rooted cuttings stayed one more week under mist (10-second burst every 10 minutes) before the final survival assessment. They were then brought to the plastic chamber.

Results and analysis.

Data recording for the shoots in the first three collections.

A summary of the data of the three shoot collections is given in Table 3.

Table 3 : Summary statistics for shoots and cuttings of the three collections.

Species	Data	Collect 1	Collect 2	Collect 3	Mean
Am15 (4)	Avg nb of shoots	4.0	4.3	1.5	4.1
	Avg length	*13.7*	*20.5*	*21.5*	*17.2*
	Avg nb of cuttings per shoot	3.9	4.0	6.5	4.0
Am21 (4)	Avg nb of shoots	4.0	2.0	2.0	3.0
	Avg length	*16.6*	*16.4*	*13.4*	*16.6*
	Avg nb of cuttings per shoot	2.9	3.3	4.3	3.3
Am24 (4)	Avg nb of shoots	2.8	3.3	3.0	3.0
	Avg length	*25.5*	*12.9*	*13.0*	*18.7*
	Avg nb of cuttings per shoot	5.4	4.0	3.3	4.0
Am6 (8)	Avg nb of shoots	3.8	3.5	3.5	3.6
	Avg length	*17.1*	*23.7*	*20.3*	*20.3*
	Avg nb of cuttings per shoot	4.2	4.0	4.8	4.3
AC (8)	Avg nb of shoots	2.5	2.0	2.5	2.3
	Avg length	*18.5*	*24.8*	*23.1*	*24.0*
	Avg nb of cuttings per shoot	3.8	4.7	4.3	4.7

In a two-way analysis of variance, no significant differences were found between clones and collections for the three characters (Number of shoots, length of the shoots, number of cuttings per shoot).

Rooting and survival rates for the first two collections.

Tables 5 and 6 give the rooting and survival rates per clone / species for the first two collections for the intermediate and terminal cuttings respectively. Table 7 shows the global results including both intermediate and terminal cuttings. More detailed information per block and clone / species is given in Appendix A.

Table 5 : Rooting and survival rates for intermediate cuttings.

	rooting rate			Surv1*			Surv2**		
	Collection 2	Collection 1	Average	Collection 2	Collection 1	Average	Collection 2	Collection 1	Average
AC	93.8%	81.3%	87.5%	95.6%	76.9%	86.9%	89.6%	62.5%	76.0%
Am6	93.1%	61.1%	77.1%	96.4%	59.1%	80.0%	75.0%	36.1%	55.6%
Am15	66.7%	24.4%	45.6%	89.5%	72.7%	83.3%	37.8%	17.8%	27.8%
Am21	45.8%	25.0%	35.4%	100.0%	83.3%	93.3%	37.5%	20.8%	29.2%
Am24	41.7%	41.7%	41.7%	83.3%	60.0%	68.8%	20.8%	25.0%	22.9%

** Surv1 gives the survival rate of rooted cuttings.*

*** Surv2 gives the global survival rate of the cuttings.*

Table 6 : Rooting and survival rates for terminal cuttings.

	rooting rate			Surv1*			Surv2**		
	Collection 2	Collection 1	Average	Collection 2	Collection 1	Average	Collection 2	Collection 1	Average
AC	66.7%	38.9%	52.8%	72.7%	42.9%	61.1%	44.4%	16.7%	30.6%
Am6	93.9%	93.9%	93.9%	71.4%	74.2%	72.9%	60.6%	69.7%	65.2%
Am15	91.7%	58.3%	75.0%	80.0%	85.7%	82.4%	66.7%	50.0%	58.3%
Am21	100.0%	83.3%	91.7%	100.0%	80.0%	90.0%	83.3%	66.7%	75.0%
Am24	91.7%	58.3%	75.0%	60.0%	42.9%	52.9%	50.0%	25.0%	37.5%

Table 7 : General rooting and survival rates.

	rooting rate			Surv1*			Surv2**		
	Collection 2	Collection 1	Average	Collection 2	Collection 1	Average	Collection 2	Collection 1	Average
AC	86.4%	69.7%	78.0%	91.1%	71.7%	82.4%	77.3%	50.0%	63.6%
Am6	93.3%	71.4%	82.4%	88.1%	65.3%	77.4%	70.5%	46.7%	58.6%
Am15	71.9%	31.6%	51.8%	86.2%	77.8%	83.0%	43.9%	24.6%	34.2%
Am21	63.9%	44.4%	54.2%	100.0%	81.3%	91.4%	52.8%	36.1%	44.4%
Am24	58.3%	47.2%	52.8%	68.8%	52.9%	60.6%	30.6%	25.0%	27.8%

The analysis of variance of the Tables 5, 6 and 7 highlights that :

1- In the overall experience, no block effect appeared.

2- Considering all the cuttings, there was a highly significant difference between clones / species for rooting. This difference still appeared within the two categories of cuttings (intermediate and terminal).

3- Once rooted, the survival rate did not depend onto the clone / species, though it was influenced by the duration of the weaning under mist.

4- Eventually, the final survival rate (surv2) depended on the rooting success, and like this latter was influenced by the clone / species.

5- For all the cuttings, between the two collections, the difference for the two variables (rooting and survival) was also very significantly in favour of the second collection (P=0.0001). The differences were still present in the two categories of cuttings, except for the survival of terminal cuttings (P=0.1718).

The differences observed between clone / species for the rooting and global survival rates were most of the time in favour of the bulks, while the single clones of *A. mangium* had lower performances. However, the better performance of the bulks was not observed within the terminal cuttings.

Within the *A. mangium* clones, the two selected clones (15 and 21) performed slightly better than the unselected one (clone 24).

Discussion

Among the three shoot collections, no significant difference was found for the length of the shoots, their number, and the number of cuttings per shoot. The stockplants were fertilised after each collection, and thus no effect on the production, due to a deterioration of physiological conditions, was found up to the third collection. Yet, though no difference was made by the analysis, we observed that *A. crassicarpa* shoots were thinner, often longer, with an internode length far above that of *A. mangium*.

Rooting and survival rates were significantly better at the second collection, compared to the first one. The increase in rooting rate at the second collection might be explained by the physiologically more responsive conditions of the shoots, once the plants had undergo the first hedging.

The increase of survival rate may partly be explained by the shorter time rooted cuttings spent in the mist chamber before their transfer to the plastic chamber. In fact, with the first collection, a high mortality was observed for rooted cuttings at the second week in the mist chamber, due to excess watering; the second collection was then transferred to the greenhouse after only one week of weaning under mist. With an earlier trasnfer, we recorded a certain amount of mortality in the greenhouse, however the global balance was better than with the two-week long stay in the weaning.

The global survival rate for *A. mangium* clones and bulk is over 40%, while the rate for *A. crassicarpa* was 64%. Kendel (1987) found a survival rate below 10% over 3950 cuttings of *A. mangium*. This result was due to the low rooting rate (around 20%). The afterward survival of rooted cuttings was around 91%. For comparison, ours was around 78%.

Regarding the high survival of terminal compared to intermediate cuttings, it must be borne in mind that we chose to keep the first well-developed leaf. Usual lower survivals recorded for terminal cuttings are due to the absence of this leaf, only the terminal young leaflet being left. Keeping the last leaf obviously made the difference.

As for the poorer rooting of intermediate compared to terminal cuttings, it can be explained by the fact that the absence of the apical bud stimulates the growth of dormant lateral buds, thus consuming resources needed for rooting.

In the same way, it was observed that terminal cuttings grew better after transfer to the plastic chamber, the terminal part being already developed, while intermediate cuttings require time to grow a new shoot from an adventive bud.

The overall rooting rates recorded for the three *A. mangium* clones were very homogeneous, around 53%. For comparison, the rooting rates for the same material under *in vitro* conditions were 25, 37.5 and 76% for clone 15, 21 and 24 respectively (Galiana, 1999).

Conclusion

In the conditions of the experiment, we obtained a satisfactory rooting rate for both species, indicating that hedge-plant management and vegetative propagation in the nursery is a suitable technique for propagationg selected material.

However, the low survival rates obtained for rooted cuttings of *A. mangium* indicate that improvement is still possible in the nursery. The low rates recorded in this experiment, though higher than those of Kendel, might be explained by some of the following points: the fragility of *A. mangium* roots, easily breakable during the transfer to polybags, and the texture of the soil that may prove being to heavy for Acacias. Actually, the soil used in Luasong nursery to fill the polybags may be the main drawback both during the transfer, where roots may be broken, and during the weaning stage, where it may slow down or prevent a good growth. A new experiment was recently set up to determine whether a lighter soil (mixed up with sand) will better suit the acacia requirements.

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Appendix A.

Details of rooting and survival rates per block and clone / species for the first two collections.

		Collection 1			Collection 2			Average collection 1&2		
	Nb of Cuttings	% Total rooting	% Total surv 1	% Total surv 2	% Total rooting	% Total surv 1	% Total surv 2	% Total rooting	% Total surv 1	% Total surv 2
Block 1										
AC	16	93.8%	80.0%	75.0%	87.5%	85.7%	75.0%	53.8%	82.8%	75.0%
Am6	24	50.0%	58.3%	29.2%	95.8%	90.9%	83.3%	72.9%	79.4%	56.3%
Am15	15	20.0%	100.0%	20.0%	66.7%	100.0%	26.7%	58.3%	100.0%	23.3%
Am21	8	50.0%	100.0%	50.0%	37.5%	100.0%	25.0%	41.7%	100.0%	37.5%
Am24	8	50.0%	50.0%	25.0%	62.5%	100.0%	50.0%	78.1%	75.0%	37.5%
Block 2										
AC	16	68.8%	72.7%	50.0%	100.0%	100.0%	100.0%	66.7%	88.9%	75.0%
Am6	24	62.5%	66.7%	41.7%	87.5%	100.0%	58.3%	50.0%	82.8%	50.0%
Am15	15	33.3%	80.0%	26.7%	60.0%	100.0%	40.0%	42.5%	90.9%	33.3%
Am21	8	12.5%	100.0%	12.5%	37.5%	100.0%	37.5%	50.0%	100.0%	25.0%
Am24	8	25.0%	100.0%	25.0%	50.0%	100.0%	12.5%	59.4%	100.0%	18.8%
Block 3										
AC	16	81.3%	76.9%	62.5%	93.8%	100.0%	93.8%	56.9%	89.3%	78.1%
Am6	24	70.8%	52.9%	37.5%	95.8%	100.0%	83.3%	54.2%	78.4%	60.4%
Am15	15	20.0%	33.3%	6.7%	73.3%	77.8%	46.7%	61.7%	66.7%	26.7%
Am21	8	12.5%	0.0%	0.0%	62.5%	100.0%	50.0%	79.2%	80.0%	25.0%
Am24	8	50.0%	50.0%	25.0%	12.5%	0.0%	0.0%	46.9%	40.0%	12.5%
Block T1										
AC	6	16.7%	0.0%	0.0%	66.7%	75.0%	50.0%	58.3%	60.0%	25.0%
Am6	11	100.0%	81.8%	81.8%	90.9%	62.5%	45.5%	95.5%	73.7%	63.6%
Am15	4	50.0%	50.0%	25.0%	100.0%	100.0%	100.0%	75.0%	83.3%	62.5%
Am21	4	100.0%	75.0%	75.0%	100.0%	100.0%	100.0%	100.0%	87.5%	87.5%
Am24	4	50.0%	100.0%	50.0%	100.0%	75.0%	75.0%	58.3%	83.3%	62.5%
Block T2										
AC	6	50.0%	33.3%	16.7%	83.3%	50.0%	33.3%	79.2%	42.9%	25.0%
Am6	11	90.9%	80.0%	72.7%	100.0%	60.0%	54.5%	87.5%	70.0%	63.6%
Am15	4	75.0%	100.0%	75.0%	100.0%	33.3%	25.0%	100.0%	66.7%	50.0%
Am21	4	75.0%	100.0%	75.0%	100.0%	100.0%	50.0%	95.5%	100.0%	62.5%
Am24	4	100.0%	0.0%	0.0%	100.0%	66.7%	50.0%	75.0%	28.6%	25.0%
Block T3										
AC	6	50.0%	66.7%	33.3%	50.0%	100.0%	50.0%	50.0%	83.3%	41.7%
Am6	11	90.9%	60.0%	54.5%	90.9%	90.0%	81.8%	83.0%	75.0%	68.2%
Am15	4	50.0%	100.0%	50.0%	75.0%	100.0%	75.0%	50.0%	100.0%	62.5%
Am21	4	75.0%	66.7%	50.0%	100.0%	100.0%	100.0%	95.5%	85.7%	75.0%
Am24	4	25.0%	100.0%	25.0%	75.0%	33.3%	25.0%	62.5%	50.0%	25.0%

Appendix 13

Acacias spp. Follow-up of the *in vitro* planting material.

Introduction

Micropropagated plantlets from the PBL[1] were brought to the LFC's mist chamber and nursery for acclimatization and further development. From October 1998 up to February 1999, with the set up of new trials (clonal trial in Taliwas, coppicing trial in Luasong), acacias spp. represented more than 60% of the material from PBL. The new production stage launched for teak has since decreased this rate, but Acacias should become an important part of the planting material in the future, with the recent agreement made between ICSB and SSSB[2].

Material and method.

Acacias clones produced in the PBL and arriving for acclimatization are brought to the mist chamber. Plantlets which had reached a satisfactory stage of development and already have a well-developed rooting system are transferred directly into polybags, while the others are left for rooting or weaning in sand beds. Between October 1998 and February 1999 clones were mainly *A. crassicarpa*, *A. mangium* and *mangium* x *auriculiformis* hybrids. Detailed information about the origin of the clones and the former selection is available through the PBL publications and papers.

Plantlets transferred directly into polybags stayed for almost 3 weeks under spraying every 10 minutes (10-second burst), before being moved to the plastic chamber. Those in sand stayed 3 weeks under spraying every 3 minutes (10-second burst) then were transferred into polybags under the same misting regime for 1 more week, and 2 additional weeks on a bed sprayed every 10 minutes. They were then transferred to the plastic chamber.

Assessments were made at every stage, to control the survival rates and see whether one stage is critical. When possible, we sometimes discriminated rooted from unrooted plantlets at their arrival in the LFC.

Results and discussion.

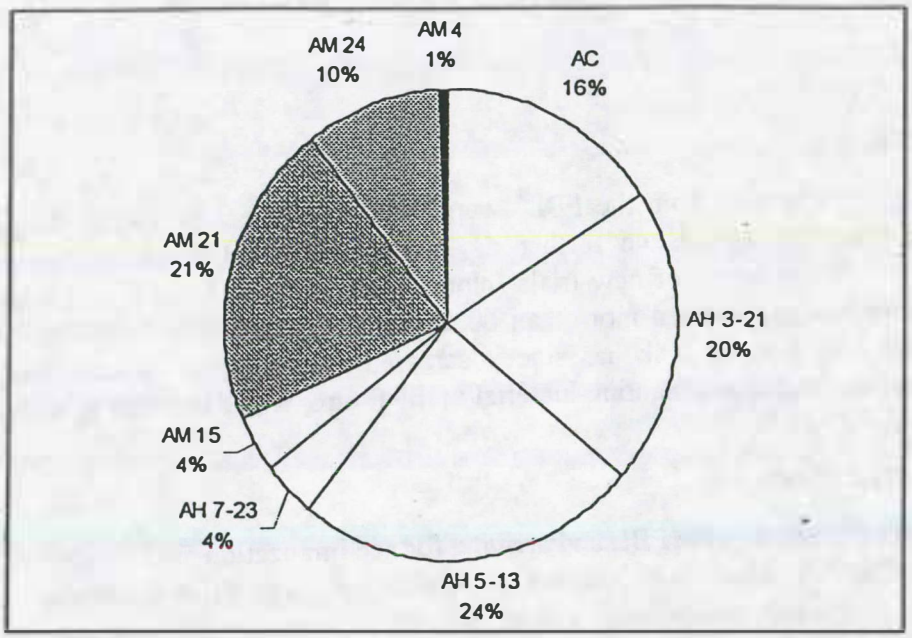
Figures 1 and 2 show the repartition of clones brought for acclimatization during the period Oct 98 - Feb 99, respectively at their arrival and after the final transfer to the plastic chamber.

At the arrival, out of 846 plantlets, acacia hybrids represented 48% of the total, while *A. mangium* clones represented 36% and *A. crassicarpa* 16%. While the percentage of

[1] Plant Biotechnology Laboratory, Tawau.
[2] Sabah Softwood Sdn Bhd, Brumas.

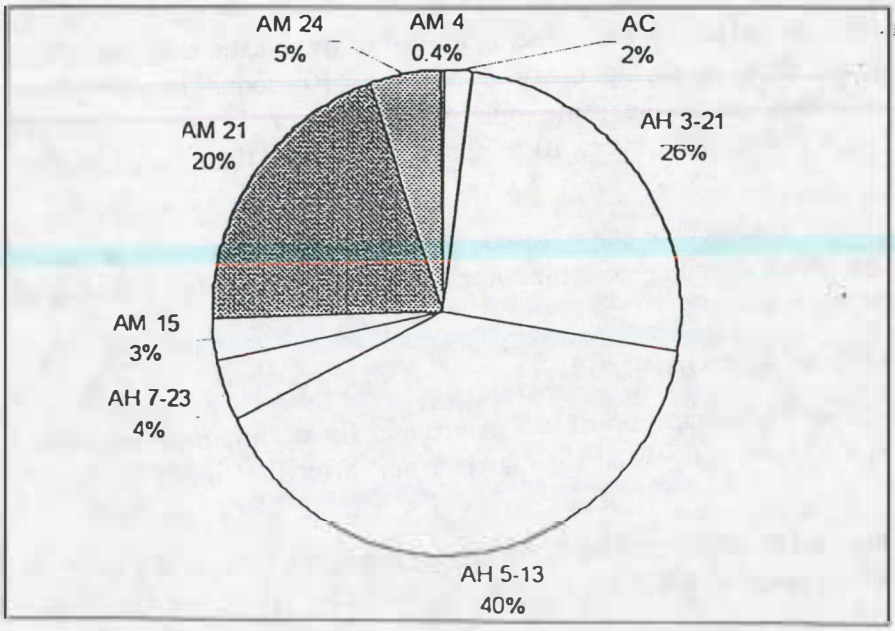
hybrids increased up to 70% of the total plants at the final transfer, *A. mangium* dropped down to 28.4% (Total of 518 plantlets).

Figure 1 : Repartition of clones from PBL at their arrival in the LFC.



Note: AM=A. mangium; AC=A. crassicarpa; AH=A. mangium x auriculiformis hybrids

Figure 2 : Repartition of the clones after their transfer to the plastic chamber.



The global survival rates to illustrate the above results are given in Table 1 per clone. The results show a great heterogeneity of survival between the clones and species. But it must be borne in mind that even the survival within the same clone may not be homogeneous, since depending on the stage of development of the plantlets at their arrival in the mist chamber. Thus, two batches of young and fragile *A. crassicarpa* plantlets all died, hence lowering the total survival rate of that particular clone. For this reason, beside the global results for the clones (Table 1), more detailed information is given in Appendix A according to the batches.

Table 1 : Survival rates of the in vitro *plantlets after acclimatization.*

Species / clone	Surv rate when transferred to polybags	Survival rate when transferred to the chamber	Global survival rate
Am 4	28.6%	100.0%	**28.6%**
Am 15	87.5%	50.0%	**43.8%**
Am 21	73.9%	79.7%	**58.9%**
Am 24	56.1%	54.3%	**30.5%**
AH 3-21	86.9%	86.3%	**76.9%**
AH 5-13	84.0%	83.0%	**82.5%**
AH 7-23	100.0%	66.7%	**66.7%**
AC	16.5%	45.5%	**7.5%**

The performance of hybrids was significantly better than that of *A. mangium* clones in term of survival. For *A. crassicarpa*, the poor results were due to the total loss of two batches (73% of the plantlets). The remaining batch yielded a survival rate at transfer into polybags of 62.9%, and a global survival rate of 28.6%.

The global survival rate of the plantlets transferred to polybags at their arrival was 84%, while that of plantlets transferred to sand beds for acclimatization was only 54%. As expected, rooted plantlets performed much better than unrooted ones (Table 2).

Table 2 : Survival rates of the in vitro *plantlets after acclimatization.*

	Surv rate when transferred to polybags	Survival rate when transferred to the chamber	Global survival rate
Rooted plantlets	100.0%	38.1%	**38.1%**
Unrooted plantlets	37.0%	5.9%	**2.2%**

For more accuracy, these results should be compared and enriched with those obtained at the nursery in Taliwas. Results are available through the Taliwas and PBL personnel.

Conclusion

In Luasong, the survival of *in vitro* plantlets has proven to be better than that of cuttings. While for cuttings the main factor for survival seems to be the rooting rate dependent on the species or clone, the main factor of survival for *in vitro* plantlets is the stage of development reached at their arrival in the mist chamber. Emphasis should be put on sending preferably rooted plantlets, since almost all the unrooted plantlets died in Luasong. Those results may be balanced by the comparison with the performances in Taliwas, where soil and overall conditions are different.

Appendix A : Detailed information about the acclimatization of the in vitro *plantlets from PBL.*

Species / clone	*Nb of plants at the arrival*		*Medium at the arrival*	*Nb of plants at transfer to polybags*		*Nb of plants at transfer to the chamber*		*Survival rates when transferred to*		*Glob surviv rate*
	R / NR	*Total*		*R / NR*	*Total*	*R / NR*	*Total*	*Polybags*	*Chamber*	
AM 4	-	7	Sand	-	2	-	2	29%	100%	29%
AM 15	16/0	16	Sand	-	12	-	12	75%	100%	75%
AM 15 5/11	8/8	16	Sand	8/8	16	1/1	2	100/100%	13/13%	13/13
AM 21	103/0	103	Sand	-	73	-	61	71%	84%	59%
AM 21 2/5	60/0	60	Sand	55/0	55	44/0	44	92%/-	80%/-	73%/
AM 21 5/11	3/14	17	Sand	3/2	5	1/0	1	100/14%	33/0%	33/0
AM 24	24/0	24	Sand	-	10	-	6	42%	60%	25%
AM 24 2/5	24/0	24	Sand	19/0	19	13/0	13	79%/-	68%/-	54%/
AM 24 5/11	10/24	34	Sand	10/7	17	6/0	6	100/29%	60/0%	60/0
AH 5-13	67/3	70	Sand	-	57	-	50	81%	88%	71%
AH 5-13	-	?	Sand	-	51	-	36	-	71%	-
AH 5-13	85/2	87	Polybags	-	-	-	84	-	97%	97%
AH 5-13	-	19	Polybags	-	-	-	10	-	53%	53%
AH 5-13 29/6	30/0	30	Sand	27/0	27	26/0	26	90%/-	96%/-	87%/
AH 3-21	44/1	45	Sand	-	41	-	39	91%	95%	87%
AH 3-21	70/1	71	Polybags	-	-	-	60	-	85%	85%
AH 3-21	-	18	Polybags	-	-	-	10	-	56%	56%
AH 3-21 29/6	39/0	39	Sand	32/0	32	24/0	24	82%/-	75%/-	62%/
AH 7-23 29/6	33/0	33	Sand	33/0	33	22/0	22	100%/-	67%/-	67%/
AC 21/7	36/15	51	Sand	-	0	-	-	0%	-	0%
AC 02/7	32/15	47	Sand	-	0	-	-	0%	-	0%
AC sdlgs 6/5	35/0	35	Sand	22/0	22	10/0	10	63%/-	45%/-	29%

Note : R/NR = rooted/unrooted plantlets.

Appendix 14

Octomeles sumatrana. Thinning trial.

Introduction

In Taliwas, two weeding and fertilizer trials were established with *Octomeles sumatrana* (binuang) in April 1995 on a 5-ha plot, and declared completed in 1997, after 2 years. Since thinning treatment has never been carried out on *Octomeles*, and no prescription is available for this species, we decided to convert the whole 5-ha plot into a thinning trial. Due to the heterogeneity of the trees in height and DBH after the different treatments applied during the previous 2 years, the first stage was to design new blocks based on the height of the trees. In Septembre 1998, we applied 3 thinning intensity. A second thinning should occur in one to two years, according to the growth.

Material and method.

The former weeding and fertiliser trials had an area of respectively 1.82 and 3.18 ha (Total = 5 ha). After two years under different treatments at different intensity, trees were very heterogeneous in height and DBH. Map 1 (Appendix A) gives the repartition of trees by height after the two trials were declared completed. Based on this map, we designed new homogeneous blocks (Map 2 – Appendix B).

Five new blocks were designed (A, B, C, D and E), and three treatments of 40, 30 and 20 % thinning intensity (in term of number of trees and not basal area) were applied (treatment 1, 2 and 3 respectively).

Three lines of buffers were left around the whole 5-ha plot, and 2 more lines around each experimental unit. A second thinning should be carried out in 1 to 2 years.

The choice of the trees to be felled down was made on the following criteria :

> To leave the better trees according to their phenotypic characters (branching, forking, straightness, DBH).
>
> To avoid to open too much the area, and find a suitable repartition of the trees that will remain.
>
> The thinning was carried out on the plots including the buffer, the thinning rate being applied to the whole experimental unit.

To allow accuracy of the analysis, the design was conceived so that after the second thinning, there will be at least 12 trees to be measured in every experimental unit (sub-blocks) After the first thinning, a new assessment of the trees was made to measure the real intensity of the thinning according to the basal area actually removed.

Data and Results

The whole 5-ha area of the trial is bordered between two roads, and is not square-shaped This constraint, and the need for designing homogeneous blocks large enough to enable a

sufficient number of trees to be measured after the two thinnings, lead us to design some blocks in a non-square shape. Therefore, some blocks are sometimes reduced to two lines of non-buffer trees in some of their part.

The results of the first thinning are given in Table 1 and Figures 1, 2 and 3. As expected, the thinning intensity based on the actual basal area removed did not match the percentage planned based on the number of trees to be removed. The statistical analysis by ANOVA on the actual percentage of basal area removed however showed no significant difference between the blocks within treatmens. Therefore, the slight difference between treatment of the "same intensity" (based on the number of trees removed – see Figure 2) can be ignored.

Table 1 : Data after the first thinning on O. sumatrana *in Taliwas*

	area (m2)	Nb Trees	Nb No Th	Nb Th	% Th planned	% Th actual	Avg BA (cm2)	BA No Th (cm2)	BA Th (cm2)	% BA Th	Avg BA Tree Th	Avg BA after Th
A1	1152	72	56	16	20%	22%	137	8824	1010	10%	63	158
A2	1632	102	71	31	30%	30%	166	12747	4217	25%	136	180
A3	1968	123	73	50	40%	41%	152	12586	6170	33%	123	172
B1	1136	71	57	14	20%	20%	172	10182	2027	17%	145	179
B2	1600	100	71	29	30%	29%	150	12024	2937	20%	101	169
B3	1904	119	73	46	40%	39%	162	13805	5490	28%	119	189
C1	1264	79	63	16	20%	20%	152	9993	2023	17%	126	159
C2	1424	89	61	28	30%	31%	156	10114	3805	27%	136	166
C3	2208	138	82	56	40%	41%	151	13863	6962	33%	124	169
D1	1136	71	57	14	20%	20%	162	10217	1287	11%	92	179
D2	1552	97	70	27	30%	28%	129	10767	1720	14%	64	154
D3	2208	138	82	56	40%	41%	154	14955	6232	29%	111	182
E1	1488	93	76	17	20%	18%	149	12464	1377	10%	81	164
E2	1520	95	69	26	30%	27%	109	8193	2203	21%	85	119
E3	2112	132	80	52	40%	39%	123	12515	3698	23%	71	156

Note : all data are given for the individual experimental units. and not for one hectare.

Legend : *Avg (average basal area) refers to the average basal area of the mean tree.*

Area	* Experimental unit area	Avg BA	* Average basal area before thinning
Nb Trees	* Number of trees	BA No Th	* Basal area of not thinned trees
Nb No Th	* Number of trees not thinned	BA Th	* Basal area of thinned trees
Nb Th	* Number of trees thinned	% BA Th	* % of the TBA removed by thinning
% Th actual	* Actual % of thinning	Avg BA Tree Th	* Average basal area of thinned trees
% Th planned	* % of thinning planned	Avg BA after Th	* Average basal area after thinning

Figure 1 : Part of the basal area removed and remaining after the thinning.

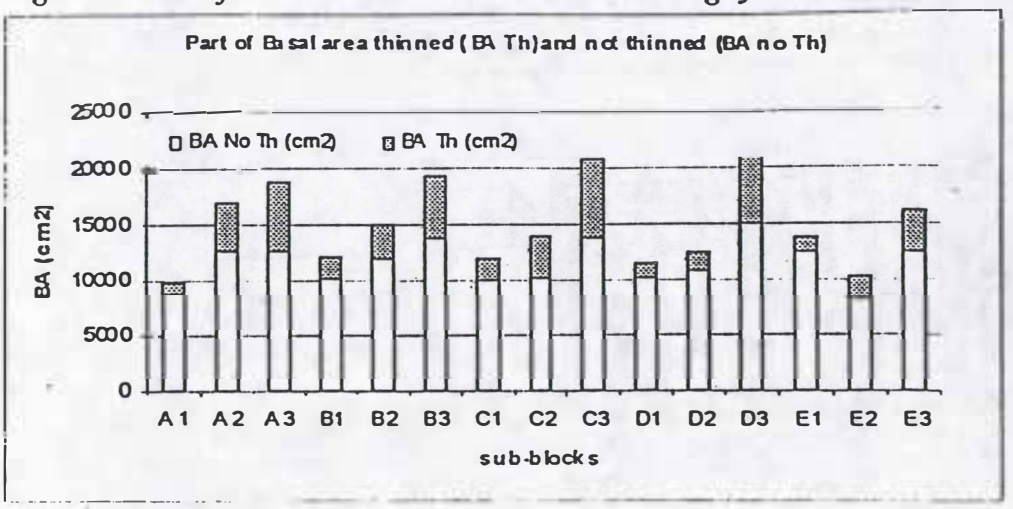
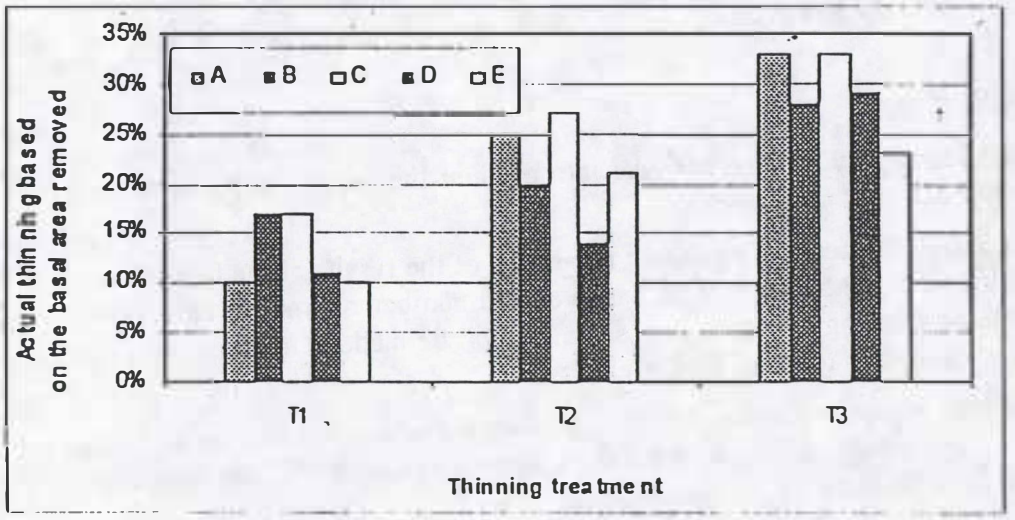
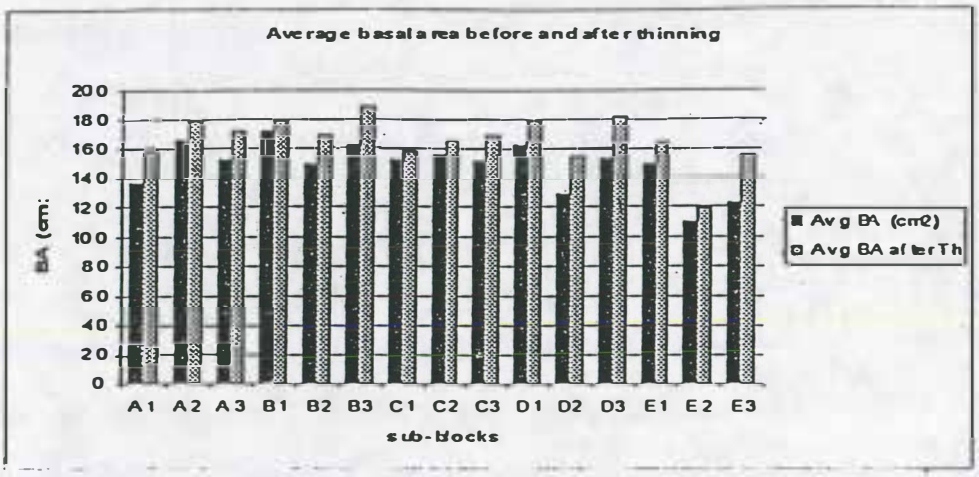


Figure 2 : Actual percentage of the basal area removed, per treatment and per block.



The average percentage of thinning calculated on the basal area removed is respectively of 13, 21.7 and 29.7% for treatment 1, 2 and 3. The most heterogeneous treatment is the treatment number 2, the actual thinning intensity ranging from less than 15% to more than 25%.

Figure 3 : Average basal area before and after thinning per sub-block.



The average increase brought by the thinning for the basal area of the mean remaining tree was 12.2% (148.3 to 166.3 cm2); it was 8.7, 11 and 17% for treatment 1, 2 and 3 respectively.

Conclusion

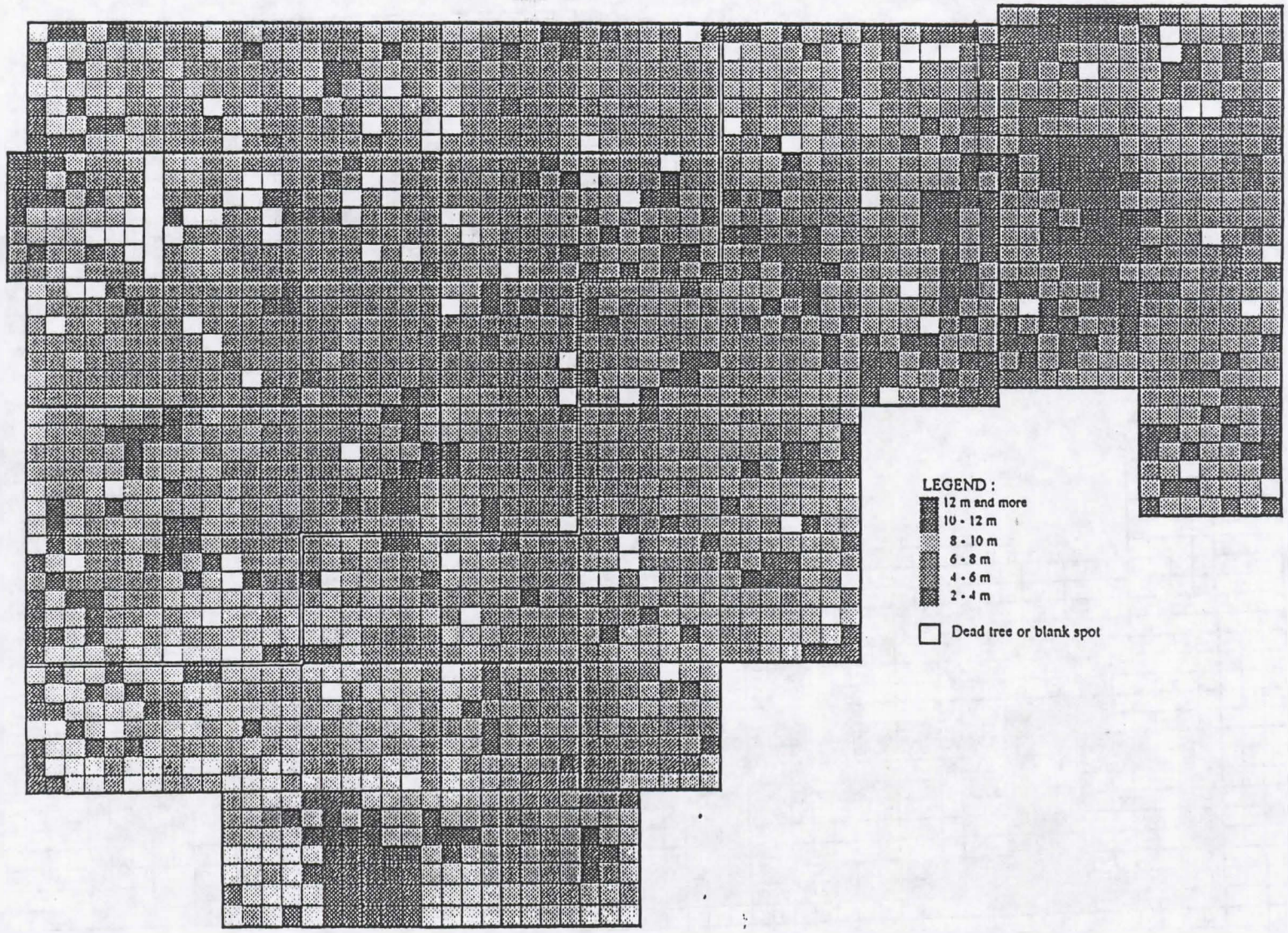
The second thinning may or may not take into account the heterogeneity in terms of basal area within treatments, as follows:

(i) The thinning intensities will be chosen regardless of the results of the first thinning, and applied with the same method, based on the number of trees to be removed. Following the thinning operation an assessment will be made to calculate the actual thinning intensities in terms of basal area.

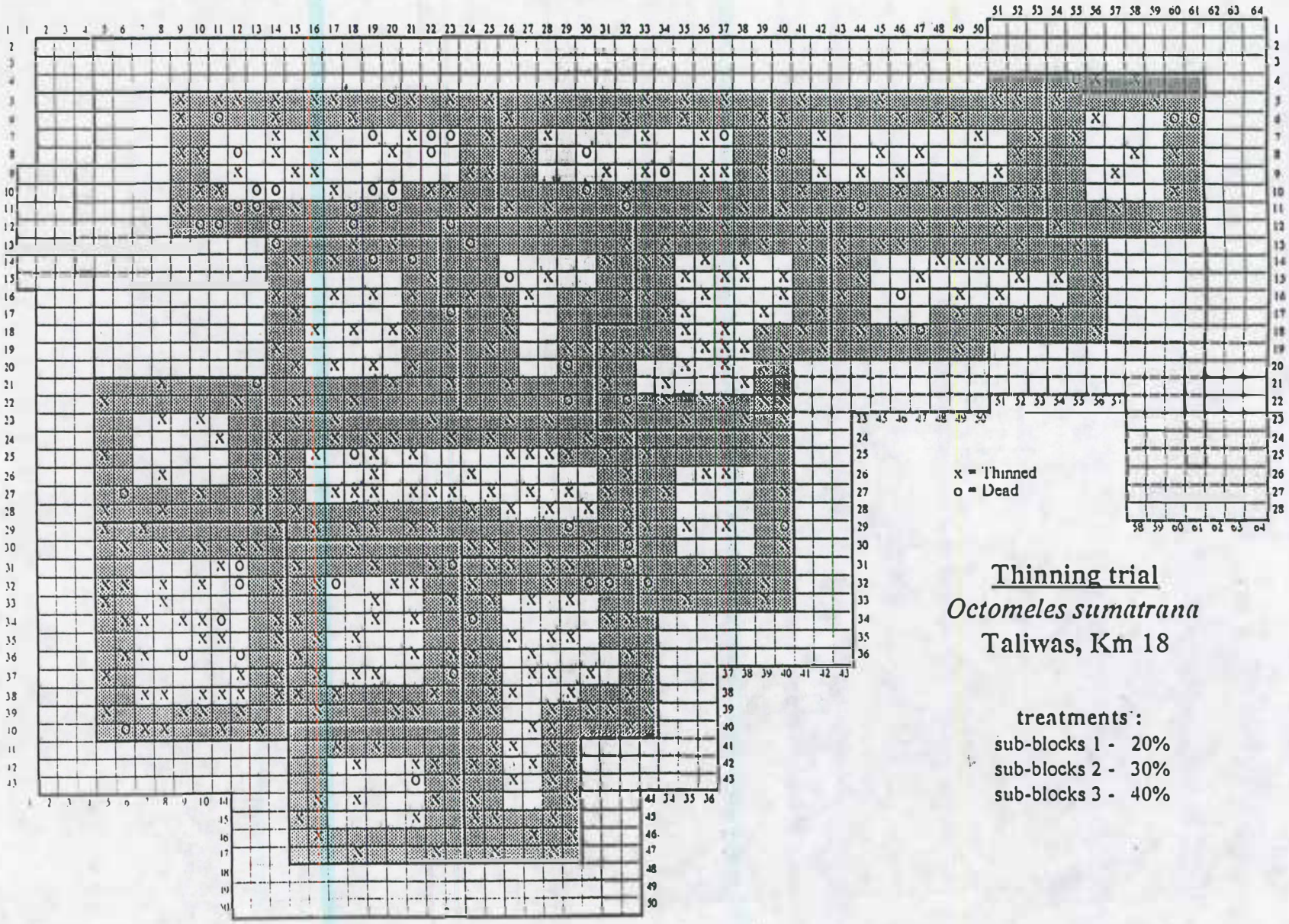
(ii) The final rate of thinning being decided, the thinning intensities will be calculated based on the number of trees to remove in order to balance the heterogeneity in basal area brought about by the first thinning.

An assessment will soon be made in April/May 1999 to see whether the growth has been boosted by the thinning. These data will allow a first comparison between the treatments and may help to schedule the next thinning operation (rates to be applied, date of the operation...), that should occur in one to two years from the first thinning.

Appendix A : Repartition of the trees by height range before thinning



Appendix B ; New experimental design and results after thinning.



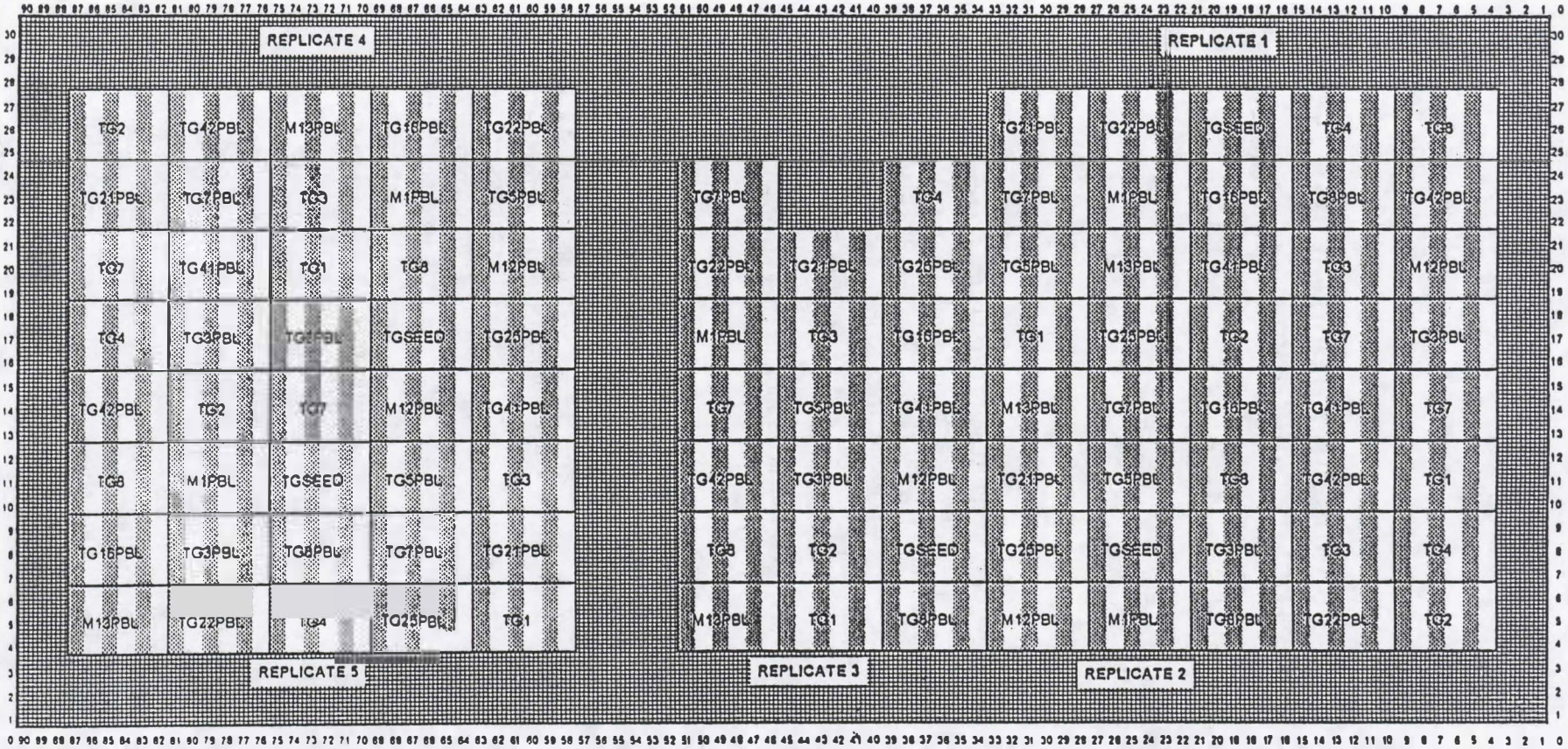
Thinning trial

Octomeles sumatrana

Taliwas, Km 18

treatments :

sub-blocks 1 - 20%
sub-blocks 2 - 30%
sub-blocks 3 - 40%



Treatments	in vitro cultures (PBL)	Cuttings (PISP)	Seedlings (PISP)
	TG5PBL	TG1	TGSEED
	TG7PBL	TG2	
	TG8PBL	TG3	
	TG16PBL	TG4	
	TG21PBL	TG7	
	TG22PBL	TG8	
	TG25PBL		
	TG41PBL		
	TG42PBL		
	M1PBL		
	M12PBL		
	M13PBL		

Design:
RCB
9-tree plots
5 replicates
Spacing 4 m x 2 m
Filler lines (one over two)

Date planted: September 1998
1st assessment:
2nd assessment:
3rd assessment:
1st thinning:
2nd thinning:

Appendix 16

Third Assessment (25 Months after planting) of the Teak Progeny / Provenance Trial in Taliwas (Sabah, Malaysia)

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Introduction

As reported in the Steering Committee Meeting Report (CIRAD-Foret / ICSB) of April 1998, a provenance / progeny trial was planted with teak in March 1997. During the first year the trial grew smoothly, essentially due to regular rainfall. In 1998, due partly to harsh weather, the trial suffered a number of biotic and abiotic stresses, including wind breakages, pest attacks and dieback. At present, a number of trees carries minor or permanent trunck defects such as a low fork, bending, heavy branching, breakages, cankers, etc. We report here the results from the third assessment of the trial at 25 months after planting.

Material and Methods

The trial was established in Taliwas (km 18) on a flat and flooded-prone area. A first assessment of the trial was carried out after 4 months, a second assessment after 15 months and the third at 25 months after planting. At the third assessment the following characters were measured: height, diameter, straightness, forking, bending, branching and breakages. Height and diameter were measured with clinometers and diameter-tape respectively. The other characters concerning tree defects were visually assigned to three classes: 0=no defects; 1=minor defects leaving a possibility to the tree to recover; 2=major permanent defect hindering the possibility for the tree to recover. Pest attacks were recorded by the Pest and Diseases Unit of ICSB.

The information delivered earlier concerning the experimental design, which was assumed to be a partially equilibrated incomplete block design (Williams & Matheson 1995) is untrue due to errors occurred at the planning-planting time. The experimental design is more accurately to be considered as a randomised complete blocking. It includes 3 replications, 41 treatments and 15 trees per experimental unit, for a total of 1.845 trees.

The treatments included 15 ~~families~~ Provenances collected in the wild from true provenances (TR-PRO), and 26 families collected in a clonal seed orchard (CSO) established with material selected in a provenance trial by CIRAD-Foret in Ivory Coast. One filler line over two was interplanted with teak within the treatments, with the objective to be the material on which to focus the first thinning. Due to the damages incurred by the trial due to heavy rainfall and winds, the filler lines were thinned in October 1998.

Information concerni ographic origin of the provenances was released earlier (Bacilieri et al. 1997). a new regroupement between provenances was made based on geographic pro: o Indians provenances (Virnoli-Gilalegundi-Karadibetta [v-g-k] and Maukal-Mas [mk-ms]) and a Thai provenances (Mae Huat-Pong Salee [mh-pg]) were repr oth in the TR-PRO and CSO material. The acronyms and countries of origin of t oups are showed in Table 1.

Table 1. Acrc countries of origin of the provenances.

India	
Chand	ur
Sakrb	bail
v-g-k	alegundi, Karadibetta
Mk-ms	sale Valley
Nb-nc	ellicutha
Purun	
Thailand	
Mh-pg	ng Salee
Hn-bp	, Ban Phai Lai
Tanzania	
Kihuw	
Mtibw	
Ivory Coast	
Ba-ko	ekro
Laos	
Pakla	
PNG	
Png	

The raw data were first studied b of summary statistics. Then. the analysis of variance of the treatments was can ased on a individual model (each tree is accounted for and the within-experim riance forms the residual) as follows:

$$Y = mean + repetition + origin + prov \ldots in\ origin + family\ within\ provenance\ within\ origin + interactions\ with \ldots ror\ term$$

Provenances chand, mtibw, pakla, p sakrb were represented by only one family; as in this case provenance a els could not be separated, these provenances had to be discarded from th is with all the treatment levels. Their ranking was analysed in a separate analys provenance effect.

As pest attacks or wind, breakages rbed the growth, the analysis of variance of the height and diameter was ily on the trees with minor or no

defects. By contrast, the variability of the trunck form was studied by another analysis taking in account all living trees.

Results

Summary statistics for the trial are given in Table 2. The survival rate was 89%, while the number of tree completely free of defects was 45%. However, we found that 37% of the trees had only minor defects and may recover in the future. Among the major permanent defects affecting 18% of the surviving trees, the most important were curvatures in the trunck, bending and low forks. By visual inspection, trunck curvatures and low forks seemed caused by one or several pest species, in particular stem borers. Possibly stem borers were also at the origin of part of the breakages and diebacks, especially by weakining the trunck at a given point and making it more sensitive to strong wind or fungal infection. Strong winds occurred in the last part of 1998, and the field staff reported that the day following one of such events, a number of trees were found freshly broken.

Table 2. Summary statistics for the provenance / progeny trial in Taliwas at 25 months after planting.

total trees planted	1845
survival	89%

	average	st. dev.	maximum
height (m)	9.82	2.1	16.0
diameter (cm)	9.88	1.8	16.7

	percentage of trees	cumul.
without defects	45%	45%
with one minor defect	26%	71%
with two minor defects	11%	82%
with one major defect	8%	
with two major defects	8%	

	Percentage of trees with defect				
note	*straight.*	*fork*	*breakage*	*Bending*	*branch.*
no defect	64%	84%	95%	83%	85%
minor defect	26%	10%	2%	10%	14%
major permanent defect	10%	6%	2%	7%	1%

Figure 1 shows the distribution of trees in terms of diameter and height. Among the smaller trees (2-5 meters tall), many were bending, forking or broken trees; these trees

were discarded from the statistical analysis of height and diameter; they have instead been kept in the analysis of the trunck form.

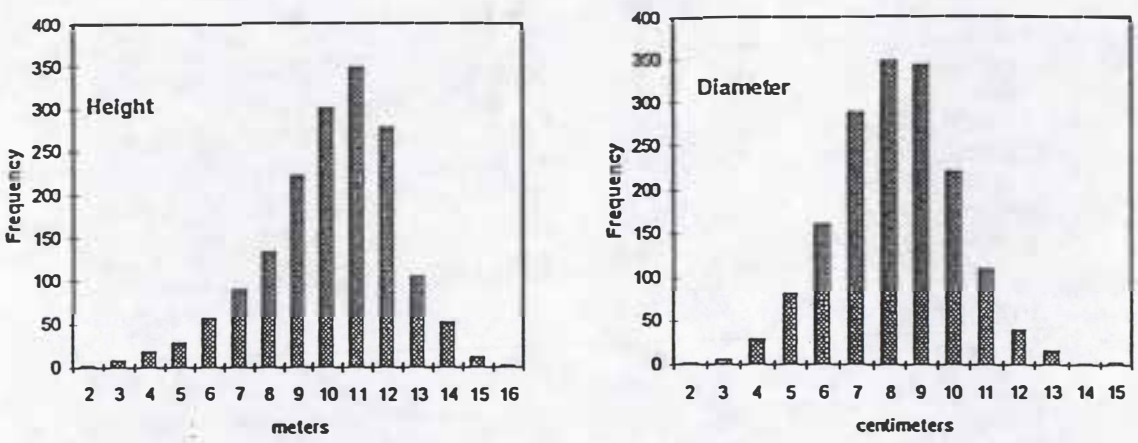

Figure 1. Distribution of trees in height or diameter classes in Taliwas at 25 months after planting.

Significant differences were found in height and diameter growth among families and origins (CSO or TR-PRO), while the provenance effect on these characters was undetectable (Table 3). The main reason of the failure to capture a provenance effect was that after the provenance regrouping, only few provenances were available within origins, and included a very variable number of families. For the characters of trunck form, significant differences were found for straightness (origin and provenance levels), bending (origin and family levels) and branching (origin level). No significant differences among treatments were found for forking and breakages (Table 3).

The ranking of the treatments provided an interesting pattern, with the selected CSO material doing consistently better than the non-selected TR-PRO material for almost all measured characters (Table 4). The CSO material was 10% taller and larger in diameter, and it was less prone to trunck defects (doing significantly better for straightness, bending and branching).

In terms of provenances, African, Indian and Thailand materials were all well represented at the top of the ranking for height, confirming the absence of a significant pattern at this level. By contrast, the significant difference in straightness between provenances can be useful for a later selection.

The family level comprised a large variability for the height, diameter and bending, indicating that important genetic gains can be obtained selecting for these characters between families (Table 4). For developmental or genetic reasons, the tallest trees were generally those with the best stem form (see for example the score for straightness compared to the mean height at both family or provenance level), that means that selecting for height will generally bring along a selection for good stem from.

Table 3. Analysis of variance on several characters measured in the Taliwas teak trial at 25 months after planting

HEIGHT 1999

Source	DF	Sum of Squares	F Value	Pr > F	Variance Component
Model	104	1082.844	3.79	0.000	
Error	1101	3024.127			2.750
REPET	2	29.520	5.37	*0.005*	0.014
TPIC	1	129.322	49.99	*0.019*	0.337
REPET*TPIC	2	5.174	0.94	0.390	0.000
PROVG(TPIC)	8	88.293	1.48	0.241	0.000
REPET*PROVG(TPIC)	16	119.491	2.72	*0.000*	0.012
FAM(PROVG*TPIC)	25	318.674	1.73	*0.049*	0.157
REPE*FAM(PROVG*TPIC)	50	367.405	2.68	*0.000*	0.398

DIAMETER 1999

Source	DF	Sum of Squares	F Value	Pr > F	Variance Component
Model	104	868.992	3.22	0.000	
Error	1101	2855.078			2.594
REPET	2	167.103	32.22	*0.000*	0.180
TPIC	1	183.590	307.89	*0.003*	0.484
REPET*TPIC	2	1.193	0.23	0.795	0.034
PROVG(TPIC)	8	21.806	0.69	0.696	0.000
REPET*PROVG(TPIC)	16	63.305	1.53	0.083	0.000
FAM(PROVG*TPIC)	25	222.981	3.04	*0.000*	0.145
REPE*FAM(PROVG*TPIC)	50	146.697	1.13	0.249	0.109

STRAIGHTNESS

Source	DF	Sum of Squares	F Value	Pr > F	Variance Component
Model	104	155.435	4.07	0.000	
Error	1312	482.145			0.365
REPET	2	22.881	31.13	*0.000*	0.037
TPIC	1	16.003	122.22	*0.008*	0.049
REPET*TPIC	2	0.262	0.36	0.700	0.000
PROVG(TPIC)	8	17.202	3.8	*0.011*	0.022
REPET*PROVG(TPIC)	16	9.049	1.54	0.079	0.000
FAM(PROVG*TPIC)	25	20.436	0.99	0.501	0.000
REPE*FAM(PROVG*TPIC)	50	41.465	2.26	*0.000*	0.038

FORK

Source	DF	Sum of Squares	F Value	Pr > F	Variance Component
Model	104	54.360	1.79	0.000	
Error	1312	383.717			0.293
REPET	2	6.433	11	*0.000*	0.010
TPIC	1	0.093	0.87	0.450	0.001
REPET*TPIC	2	0.214	0.37	0.693	0.000
PROVG(TPIC)	8	2.316	0.44	0.881	0.000
REPET*PROVG(TPIC)	16	10.570	2.26	*0.003*	0.002
FAM(PROVG*TPIC)	25	12.028	1.18	0.300	0.000
REPE*FAM(PROVG*TPIC)	50	20.327	1.39	*0.039*	0.012

Table 3. (*continued*)

BREAKAGE

Source	DF	Sum of Squares	F Value	Pr > F	Variance Component
Model	104	17.960	1.55	0.001	
Error	1312	146.407			0.112
REPET	2	0.582	2.61	0.074	0.001
TPIC	1	0.076	6.23	0.130	0.000
REPET*TPIC	2	0.024	0.11	0.897	0.000
PROVG(TPIC)	8	0.752	1.33	0.297	0.000
REPET*PROVG(TPIC)	16	1.130	0.63	0.859	0.001
FAM(PROVG*TPIC)	25	5.660	1.24	0.251	0.002
REPE*FAM(PROVG*TPIC)	50	9.096	1.63	*0.004*	0.002

BENDING

Source	DF	Sum of Squares	F Value	Pr > F	Variance Component
Model	104	69.088	2.31	0.000	
Error	1312	377.125			0.287
REPET	2	0.106	0.19	0.831	0.000
TPIC	1	10.110	55.81	*0.018*	0.026
REPET*TPIC	2	0.362	0.63	0.533	0.000
PROVG(TPIC)	8	4.823	2.2	0.086	0.012
REPET*PROVG(TPIC)	16	4.390	0.95	0.505	0.000
FAM(PROVG*TPIC)	25	18.971	1.83	*0.035*	0.000
REPE*FAM(PROVG*TPIC)	50	20.768	1.44	*0.024*	0.010

BRANCHING

Source	DF	Sum of Squares	F Value	Pr > F	Variance Component
Model	104	42.089	3.1	0.000	
Error	1312	171.184			0.131
REPET	2	5.037	19.3	*0.000*	0.005
TPIC	1	0.379	15.45	0.059	0.000
REPET*TPIC	2	0.049	0.19	0.829	0.001
PROVG(TPIC)	8	5.677	2.22	0.084	0.005
REPET*PROVG(TPIC)	16	5.126	2.46	*0.001*	0.000
FAM(PROVG*TPIC)	25	5.125	0.5	0.967	0.000
REPE*FAM(PROVG*TPIC)	50	20.358	3.12	*0.000*	0.020

Note: Statistical effects: REPET=Repetition; TPIC=Origin (True Provenance or Clonal Seed Orchard CIRAD-Foret-IC); REPET*TPIC=Interaction; PROVG(TPIC)=Provenance within Origin; REPET*PROVG(TPIC)=Interaction; FAM(PROV*TPIC)=Family within Provenance within Origin; REPE*FAM(PROV*TPIC)=Interaction; Error=Statistical Error of the Individual Model. DF=Degrees of Freedom. F= F-test of significance of differences. Pr>F=Probability that the hypothesis of equality between treatments is true (if P=0.05, then treatments are different at 95% probability).

A robust ranking obtained through the complete statistical analysis of the trial (Table 4) is the basis for any selection to be carried out on this material. However a more complete ranking could be obtained by adding the provenances represented by only one family, which

Table 4. Means, standard deviations and significance levels of differences among treatments of all measured characters in Taiwas at 25 months after planting, ranked by height

TREATMENTS					HEIGHT		DIAMETER		STRAIGHTNESS		FORK		BREAKAGE		BENDING		BRANCHING	
	Provenance	True Prov. or Clonal Seed Orchard	Family	Number of Trees	mean	st.dev	mean	st.dev	mean	st.dev	mean	st.dev	mean	st.dev	mean	st.dev	mean	st.dev
Family ranking (height)	v_g_k	CSO	9443	42	11.83	0.26	11.38	0.25	0.31	0.09	0.27	0.08	0.05	0.05	0.02	0.08	0.11	0.05
	kihuw	CSO	9412	35	11.24	0.28	10.35	0.27	0.28	0.10	0.38	0.09	0.03	0.05	0.19	0.09	0.31	0.06
	hn_bp	CSO	9439	41	11.15	0.26	10.66	0.25	0.25	0.09	0.20	0.08	0.00	0.05	0.14	0.06	0.19	0.06
	nb_nc	CSO	9437	38	11.13	0.28	10.62	0.27	0.34	0.10	0.05	0.09	0.05	0.05	0.20	0.06	0.17	0.06
	mh_pg	CSO	9430	35	11.03	0.28	10.93	0.27	0.14	0.10	0.22	0.09	0.08	0.06	0.06	0.09	0.15	0.06
	nb_nc	CSO	9445	40	10.98	0.26	10.46	0.26	0.19	0.09	0.12	0.08	0.02	0.05	0.17	0.08	0.12	0.06
	ba_ko	CSO	9416	34	10.83	0.29	9.84	0.26	0.54	0.09	0.27	0.08	0.07	0.05	0.20	0.06	0.24	0.06
	nb_nc	CSO	9435	39	10.80	0.27	10.48	0.26	0.40	0.09	0.16	0.08	0.02	0.05	0.09	0.06	0.09	0.06
	kihuw	CSO	9431	34	10.80	0.29	10.02	0.28	0.33	0.10	0.21	0.09	0.08	0.05	0.12	0.09	0.26	0.08
	nb_nc	CSO	9418	44	10.78	0.25	9.91	0.24	0.11	0.09	0.16	0.08	0.00	0.05	0.04	0.08	0.11	0.05
	nb_nc	CSO	9429	38	10.69	0.28	10.38	0.27	0.46	0.09	0.14	0.08	0.00	0.05	0.14	0.08	0.09	0.05
	ba_ko	CSO	9483	41	10.65	0.26	10.71	0.25	0.16	0.09	0.18	0.08	0.07	0.05	0.05	0.06	0.16	0.05
	nb_nc	CSO	9434	37	10.47	0.27	10.06	0.26	0.24	0.09	0.22	0.08	0.10	0.05	0.14	0.08	0.14	0.06
	nb_nc	CSO	9440	35	10.46	0.28	10.65	0.27	0.45	0.09	0.29	0.08	0.17	0.05	0.15	0.08	0.15	0.06
	v_g_k	TR-PRO	8832	30	10.31	0.31	9.76	0.30	0.66	0.10	0.25	0.09	0.06	0.06	0.37	0.09	0.36	0.06
	v_g_k	TR-PRO	8824	30	10.28	0.31	9.73	0.30	0.76	0.10	0.50	0.09	0.20	0.05	0.17	0.08	0.42	0.06
	v_g_k	TR-PRO	8831	29	10.15	0.31	10.01	0.30	0.59	0.10	0.10	0.09	0.06	0.05	0.30	0.09	0.19	0.06
	mk_ms	CSO	9452	35	10.07	0.29	10.29	0.28	0.69	0.09	0.31	0.08	0.00	0.05	0.33	0.08	0.21	0.06
	mk_ms	CSO	9459	33	10.04	0.29	10.42	0.28	0.73	0.09	0.24	0.08	0.10	0.05	0.21	0.08	0.12	0.06
	nb_nc	CSO	9417	34	10.02	0.29	9.59	0.28	0.24	0.10	0.14	0.09	0.00	0.06	0.04	0.09	0.09	0.06
	v_g_k	CSO	9446	29	10.00	0.31	10.50	0.30	0.29	0.10	0.32	0.09	0.14	0.05	0.35	0.09	0.19	0.06
	v_g_k	TR-PRO	8833	34	9.98	0.29	8.94	0.28	0.81	0.09	0.17	0.08	0.10	0.05	0.41	0.08	0.11	0.05
	hn_bp	CSO	9456	31	9.80	0.30	10.14	0.29	0.53	0.10	0.40	0.09	0.00	0.05	0.32	0.09	0.03	0.06
	mk_ms	TR-PRO	8836	30	9.79	0.32	9.55	0.31	0.58	0.11	0.07	0.10	0.05	0.06	0.26	0.09	0.17	0.06
	nb_nc	CSO	9442	40	9.77	0.26	10.43	0.26	0.21	0.09	0.12	0.06	0.12	0.05	0.02	0.06	0.19	0.06
	mk_ms	TR-PRO	8842	29	9.74	0.31	9.04	0.30	0.58	0.10	0.28	0.09	0.14	0.06	0.36	0.09	0.06	0.06
	v_g_k	CSO	9450	41	9.71	0.28	9.82	0.25	0.33	0.09	0.16	0.08	0.07	0.05	0.04	0.08	0.07	0.06
	mk_ms	TR-PRO	8838	32	9.63	0 30	9.25	0.29	0.76	0.09	0.28	0.06	0.14	0.05	0.49	0.08	0.14	0.06
	mh_pg	CSO	9432	36	9.62	0.28	9.74	0.27	0.49	0.09	0.17	0.08	0.12	0.05	0.37	0.06	0.21	0.06
	mk_ms	TR-PRO	8839	27	9.60	0.32	9.87	0.32	1.03	0.09	0.22	0.06	0.10	0.05	0.68	0.08	0.05	0.06
	mh_pg	TR-PRO	8668	36	9.51	0.28	9.25	0.27	0.37	0.09	0.33	0.06	0.04	0.05	0.35	0.06	0.29	0.08
	mk_ms	TR-PRO	8844	33	9.44	0.29	8.98	0.29	0 56	0.09	0.32	0.06	0.00	0.05	0.24	0.06	0.04	0.06
	nb_nc	CSO	9411	34	9.31	0.29	10.30	0.29	0 25	0.09	0.40	0.08	0.33	0.05	0.12	0.08	0.09	0.08
	mk_ms	TR-PRO	8835	22	8.80	0.36	9.79	0.35	0.70	0.10	0.37	0.09	0.08	0.06	0.80	0 09	0.06	0.06
	mk_ms	TR-PRO	8841	30	8 62	0.31	8.05	0.30	0.68	0.10	0.08	0.09	0.05	0.06	0.32	0.09	0.18	0.06
	Significance of differences among families					0.049		0.000		ns		ns		ns		0.035		ns
Provenance ranking (h)	kihuw	CSO		69	11.02	0.20	10.19	0.20	0 29	0.07	0.28	0.06	0.04	0.04	0.18	0.06	0.29	0.04
	ba_ko	CSO		75	10.74	0.20	10.17	0.19	0.35	0.07	0.23	0.06	0.07	0.04	0.12	0.06	0.20	0.04
	v_g_k	CSO		112	10.52	0.16	10.57	0.15	0.31	0.05	0.26	0.05	0.06	0.03	0.14	0.05	0.13	0.03
	hn_bp	CSO		72	10.48	0.20	10.40	0.19	0.39	0.07	0.30	0.06	0.00	0.04	0.23	0.06	0.11	0.04
	nb_nc	CSO		377	10.44	0.09	10.29	0.08	0.29	0.03	0.18	0.03	0.06	0.02	0.11	0.03	0.12	0.02
	mh_pg	CSO		71	10.32	0.20	10.33	0.19	0.31	0.07	0.19	0.06	0.10	0.04	0.21	0.06	0.16	0.04
	v_g_k	TR-PRO		123	10.18	0 15	9.81	0.15	0.71	0.05	0.26	0.04	0.10	0.03	0.31	0.04	0.27	0.03
	mk_ms	CSO		68	10.06	0.20	10.36	0.20	0.71	0.07	0.28	0.06	0.05	0.04	0.27	0.06	0.17	0.04
	mh_pg	TR-PRO		63	9.51	0.28	9.25	0.27	0 37	0.09	0.33	0.06	0.04	0.05	0.35	0.06	0.29	0.06
	mk_ms	TR-PRO		203	9.37	0.12	9.22	0.12	0.70	0.04	0.23	0.03	0.06	0.02	0.45	0.03	0.10	0.02
	Significance of differences among provenances					ns		ns		0.001		ns		ns		0.066		0.064
Origin ranking (h)	*CSO*			*844*	*10.51*	*0.07*	*10.33*	*0.07*	*0.38*	*0.02*	*0.24*	*0.02*	*0.06*	*0.01*	*0.18*	*0.02*	*0.17*	*0.01*
	TR-PRO			*362*	*9.69*	*0.11*	*9.36*	*0.11*	*0.60*	*0.04*	*0.27*	*0.03*	*0.07*	*0.02*	*0.37*	*0.03*	*0.22*	*0.02*
	Significance of differences among origins					0.019		0.032		0.006		ns		ns		0.016		0.059

Note CSO=Clonal Seed Orchard (Ivory Coast), TR-PRO True Provenances (collected in the wild); Significance level: not significant=ns, significant<0.05, very significant<0.01.

were discarded for the global analysis. The analysis of the dataset containing all the families was carried out with a model similar to the previous one, but excluding the provenance effect that was generally not significant. Results are given in Table 5. The differences between treatments (origin and family) became more significant by this analysis (p=0.009 and 0.0001 for height and diameter respectively at the family level), because there were more families and no degrees of freedom were captured by the provenance level. Our former statement concerning correlation between height and stem form also applies here.The formal correlation analysis can be carried out on a later stage, once the damages to the trunck have stabilised.

Table 5. Average height and diameter (ranked by height) of all families in Taliwas. at 25 months after planting.

			Height		Diameter	
Family (average n=40)	Provenance	True Prov. or Clonal Seed Orchard	Mean	St. Err	Mean	St. Err
9443	v_g_k	CSO	11.73	0.29	11.30	0.25
9439	lm_bp	CSO	10.99	0.30	10.58	0.25
9437	nb_nc	CSO	10.97	0.31	10.49	0.26
9426	mtibw	CSO	10.88	0.30	10.82	0.26
9430	mh_pg	CSO	10.83	0.32	10.79	0.27
9445	nb_nc	CSO	10.80	0.30	10.38	0.26
9412	kihuw	CSO	10.77	0.31	10.30	0.26
9418	nb_nc	CSO	10.76	0.29	9.91	0.25
9435	nb_nc.	CSO	10.57	0.30	10.38	0.25
9431	kihuw	CSO	10.49	0.31	9.91	0.27
9416	ba_ko	CSO	10.38	0.30	9.55	0.26
9463	ba_ko	CSO	10.34	0.29	10.60	0.25
PNG	png	TR-PRO	10.32	0.30	10.67	0.25
9440	nb_nc	CSO	10.28	0.31	10.58	0.26
9429	nb_nc	CSO	10.25	0.29	10.10	0.25
9434	nb_nc	CSO	10.03	0.30	9.92	0.26
9417	nb_nc	CSO	9.96	0.33	9.60	0.28
8823	sakrb	TR-PRO	9.94	0.32	9.97	0.27
8832	v_g_k	TR-PRO	9.86	0.32	9.63	0.28
8824	v_g_k	TR-PRO	9.79	0.31	9.47	0.26
9450	v_g_k	CSO	9.66	0.30	9.91	0.25
9442	nb_nc	CSO	9.65	0.30	10.42	0.25
9452	mk_ms	CSO	9.58	0.30	10.11	0.26
8831	v_g_k	TR-PRO	9.56	0.32	9.71	0.27
9459	mk_ms	CSO	9.56	0.30	10.32	0.26
8836	mk_ms	TR-PRO	9.50	0.34	9.46	0.29
9458	lm_bp	CSO	9.39	0.31	10.01	0.27
9454	pakla	CSO	9.35	0.34	10.19	0.29
9446	v_g_k	CSO	9.30	0.32	10.15	0.27
8833	v_g_k	TR-PRO	9.23	0.29	8.71	0.25
9432	mh_pg	CSO	9.18	0.30	9.63	0.25
8367	chand	TR-PRO	9.15	0.32	7.90	0.28
9411	nb_nc	CSO	9.10	0.31	10.27	0.26
9457	purum	CSO	9.02	0.32	9.93	0.27
8842	mk_ms	TR-PRO	8.92	0.32	8.76	0.28
8668	mh_pg	TR-PRO	8.91	0.30	8.98	0.25
8838	mk_ms	TR-PRO	8.89	0.30	9.24	0.25
8844	mk_ms	TR-PRO	8.85	0.30	8.84	0.26
8839	mk_ms	TR-PRO	8.85	0.30	9.72	0.26
8841	mk_ms	TR-PRO	8.08	0.33	7.88	0.28
8835	mk_ms	TR-PRO	7.75	0.34	8.99	0.29
	Average	*CSO (n=1064)*	*10.16*	*0.06*	*10.24*	*0.05*
	Average	*TR-PRO (n=587)*	*9.20*	*0.09*	*9.22*	*0.07*

Conclusion

Summarising, by the differences among selected (CSO) and non-selected (TR-PRO) materials, and the remaining differences between families, this trial proved that genetic selection on teak can bring significant improvements in both growth and stem form.

The next works to carry out on this trial and on its relative in Luasong (Bacilieri et al., 1997) should be the analysis of the genetics and early selection of wood quality, of the genetic correlations among characters and of the genetic gains through selection.

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Appendix 17

Vegetative Propagation of *Xylia xylocarpa* – Effect of Cutting Orders on Rooting

By

David Alloysius, Thomas Roling, A. Ramsah Mahalan
Plant Improvement & Seed Production (PISP)
February 1999

Introduction

Xylia xylocarpa (Robx.) Taubert is the only *Xylia* species occurs in South-East Asia, whereas other species occur in tropical Africa and Madagascar. *Xylia* comprises 12 species all together. *X. xylocarpa* occurs naturally in India, Myanmar, Indo-China and Thailand. The hard and durable wood of this species (880-1330 kg/m^3 at 15% MC) is used for heavy construction, e.g. for posts and flooring, bridges, marine piling, railway sleepers, boat construction, furniture, etc. The railway sleepers made from untreated wood of *X. xylocarpa* could last for 12 years in Thailand and for 20-24 years in India.

X. xylocarpa has been planted as one of the potential high-value timber species for line-planting at Luasong. The species has been tested in both open-planting and line-planting under logged-over forest, but only the open-planted plot showed good growth performances (HMAI and DMAI of 2.77 m/yr and 2.81 cm/yr respectively). The oldest plot was planted in September 1990. This species coppices easily but no trial was ever made to evaluate its rooting ability for production by vegetative propagation. Seed generally quite difficult to acquire, the last supplies were done through the ASEAN Tree Seed Centre at Muak Lek, Thailand.

Objectives

1. To develop cloning technique
2. To determine effect of cutting order on rooting rate
3. To observe rooting performance of cuttings

Materials and Methods

Source of cuttings

Shoots were collected from stock plants planted in 12" x 15" polybags. These stock plants were originated from coppices of 7-year-old trees, felled during thinning operation. The original stand was established using seedlot supplied by the ASEAN Tree Seed Centre, Thailand. The stock plants have been hedged a month before the collection to produced homogenous young shoots. Only shoots that 'hard' enough (greenish in colour) were selected for the experiment.

Thirty-six shoots with at least 3 orders (nodes) were collected in all. Figure 1 illustrates the location of orders along each shoot.

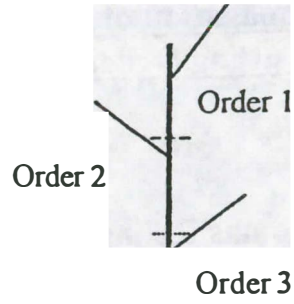


Figure 1: Location of orders within a shoot

Management of cuttings

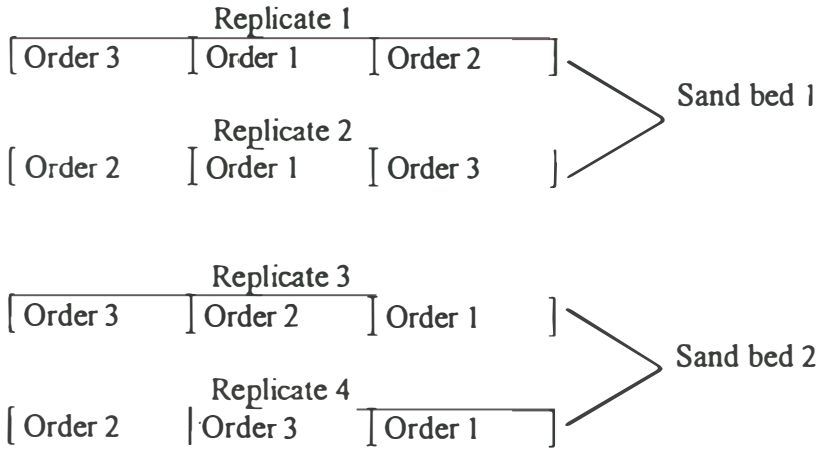
The collecting of shoots was conducted early in the morning and dipped directly into a bucket of water to reduce drying stress. The following steps are common for making cuttings at Luasong:

The cuttings are submerged into a fungicide (Thiram®) solution
The leaf surface area is reduced to about half
The basal part of the cuttings is dipped into hormone powder (Seradix 3®), excessive powder is removed by shaking the cutting lightly

- The cuttings are laid on sterilized sand beds, according to the layout design
- Timer-controlled misting system is set to spray 10-second mist at interval of 5 minutes
- The surface of the sand beds is sprayed with fungicide (Thiram®) every three days, to minimize fungus contamination.

Layout design

Randomized Complete Block Design
Treatment = Order of cuttings
4 replicates (in two sand beds)
9 cuttings per experimental unit (3x3)
Total number of cuttings = 4 reps x 3 orders x 9 = 108



Assessments

The status of rooting was assessed every two weeks. Cutting is considered rooted if it has a root of at least 2 cm length. The rooted cuttings were transferred into polybags, weaned and finally brought to the main nursery for further tending.

Results and Discussion

The first rooting occurred between the second and the fourth week after setting on the misting bed. The average rooting percentage after 12 weeks was lower (48%) compared to one showed by *Khaya ivorensis* (91%) (see report VPKIV1/98). The lower rooting percentage is expected as the stock plants were originated from a 7 years old stand, compared to from seedlings for the *K. ivorensis*. Table 1 summarized the rooting rate according to orders.

Table 1: Rooting rate of cuttings by order

Order	Number of rooted cuttings after					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
1	0	12 (33%)	23 (64%)	24 (67%)	24 (67%)	24 (67%)
2	0	4 (11%)	13 (36%)	16 (44%)	17 (47%)	17 (47%)
3	0	3 (8%)	8 (22%)	9 (25%)	10 (28%)	11 (31%)
Mean	0	17%	41%	45%	47%	48%

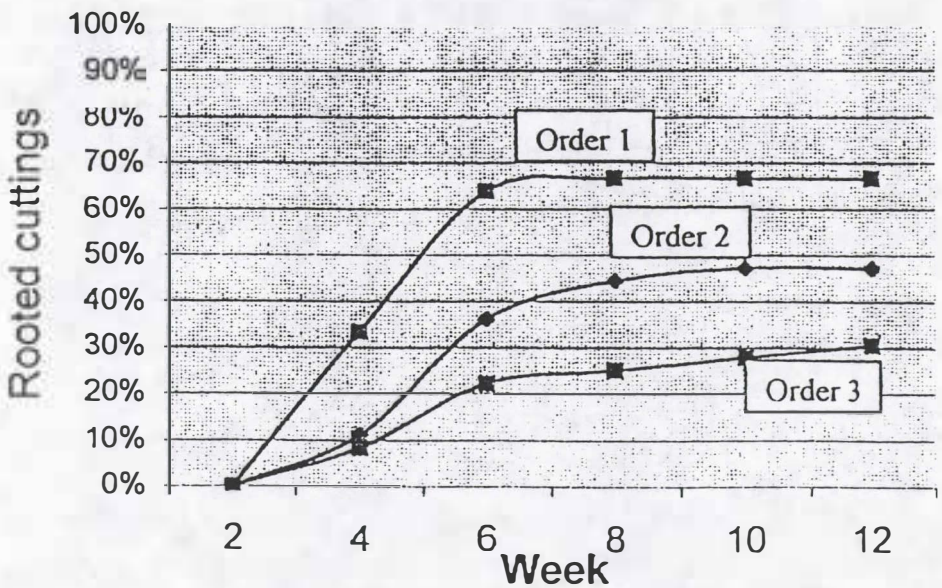


Figure 2: Rooting percentage of cuttings

Figure 2 shows that the optimal rooting period was between the 6th and 8th week after setting on the misting bed, as the rooting percentages reached their maximum and maintained thereafter. The rooting success decreased by increment of distance of cuttings from the shoots (Order 3 is the farthest from the shoot).

Analysis of variance (Table 2) shows significant differences in rooting performance among cuttings taken from different orders starting from the 6th week. There was no blocking (replicate) effect, which indicates homogeneity on the misting beds. One way ANOVA (not shown) confirms similar trend of variation with significance of F varied between 0.013 to 0.016 after the 4th week.

Table 2: Analysis of variance (ANOVA)

Main effects	Significance of F at				
	Week 4	Week 6	Week 8	Week 10	Week 12
Replicate	0.793 ns	0.622 ns	0.696 ns	0.678 ns	0.608 ns
Order	0.205 ns	0.038 *	0.037 *	0.036 *	0.034 *

Note: ns = not significant at 0.05 level
* = significant at 0.05 level

Duncan Multiple Range test indicates obvious variation between Order 1 and others as at the 6th week. However, at 8th week and after, two homogenous subsets were identified i.e. subset 1 (Orders 1 and 2) and subset 2 (Orders 2 and 3). This means that the variation was only between Order 1 and Order 3 but not between Order 1 and Order 2 or between Order 2 and Order 3.

Table 3: Duncan ranking of order effect on rooting rate

Order	Duncan subsets for α=0.05				
	Week 4	Week 6	Week 8	Week 10	Week12
1	A	A	A	A	A
2	A	B	AB	AB	AB
3	A	B	B	B	B

Note: Different letters within a column indicate significant different at α=0.05

Recommendation and Conclusion

The recommended period for rooting of cuttings on the misting bed is between six to eight weeks. There will be no beneficial effect on rooting even if the cuttings are kept longer than this period, as the rooting percentage stabilized thereafter (Figure 2).

Order 3 produced the lowest number of rooted cuttings (only 25% rooted after 8 weeks), therefore should be excluded if space on misting bed is limiting during production of cuttings.

The age of shoots for making cuttings could probably contributes in increasing the rooting rate but the exact timing is yet to be determined. Since the Order 3 does not produce good rooting, shoots could be collected at younger stage i.e. at stage when only the first and second nodes are established. This will be tested on the next trial.

Another possibility to increase the rooting performance is by making the sources of cuttings more 'juvenile' by repeated cutting-and-plant. Stock plants established from several generations of cutting-and-plant generally produce easy-to-root cuttings compare to cuttings directly taken from coppices of older trees in the field.

The finding of this study shows that cloning of mature *X. xylocarpa* is possible through conventional vegetative propagation method. Once good trees are identified, the trees could be felled for production of coppices that later to be converted as stock plants in the nursery. Mass production from these selected genotypes could be initiated when necessary.

Future trials:

1. Determination of optimal size and age of shoots for optimal rooting of cuttings.
2. Inducement of coppices by methods other than felling the trees (partial girdling, mound layering).

Appendix 18

Effect of Order on Rooting of *Khaya ivorensis* Cuttings

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Plant Improvement & Seed Production (PISP)
October 1998

Objectives

1. To determine the effect of cutting order on rooting rate
2. To observe rooting performance

Materials and Methods

Source of cuttings

Shoots were collected from stock plants originated from bulk of seedlings. The stock plants have been hedged a month before the collection to produced homogenous young shoots. Only shoots that 'hard' enough (greenish in colour) were selected for the experiment.

Thirty-six shoots with at least 3 orders were collected in all. Figure 1 illustrates the location of orders along each shoot.

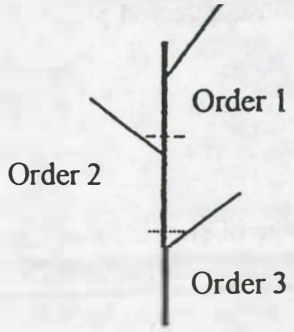


Figure 1. Location of orders within a shoot

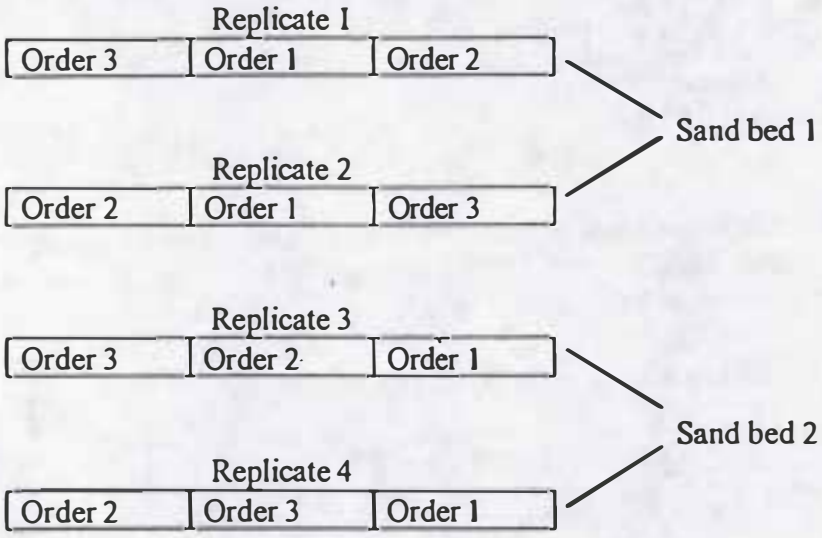
Management of cuttings

The collecting of shoots was conducted early in the morning and the collected shoots were dipped directly into a bucket of water to reduce drying stress. The following steps are the common procedures for making cuttings in Luasong:

- The cuttings are submerged into a fungicide (Thiram®) solution
- The basal part of the cuttings is dipped into hormone powder (Seradix 3®), excessive powder is removed by shaking the cutting lightly
- The cuttings are laid on sterilized sand beds, according to the layout design
- Timer-controlled misting system is set to spray 10-second mist at interval of 5 minutes
- The surface of the sand beds is sprayed with fungicide (Thiram®) every three days, to minimize fungus contamination.

Layout design

Randomized Complete Block Design
Treatment = order
4 replicates (in two sand beds)
9 cuttings per experimental unit (3x3)
Total number of cuttings = 4 reps x 3 orders x 9 = 108



Assessments

The status of rooting was assessed every two weeks. Cutting is considered rooted if it has a root of at least 2cm length. The rooted cuttings were transferred into polybags, weaned and finally brought to the main nursery for further tending (the identification of the cutting's order is maintained for further growth observations).

The experiment was concluded after 12 weeks when almost all cuttings were rooted.

Results

The cuttings started to root after two weeks on the sand beds. The average rooting percentage after 12 weeks is 91%. The rooting rate according to order of cuttings is presented in Table 1.

Table 1: Rooting rate of cuttings by order

Order	Number of rooted cuttings after					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
1	0	26 (72%)	31 (86%)	33 (92%)	33 (92%)	34 (94%)
2	0	20 (56%)	24 (67%)	27 (75%)	28 (78%)	33 (92%)
3	0	13 (36%)	25 (69%)	28 (78%)	29 (81%)	31 (86%)
Mean	0	55%	74%	82%	84%	91%

The rooting rate of Order 1 was slightly better than Order 2 and 3. Analysis of variance (Table 2) showed that the differences is only significant ($\alpha = 0.05$) at week 6, but not before and after. There was no block (replicate) effect, confonning that the environment condition is homogenous within and between the sand beds.

Table 2: Analysis of variance (ANOVA)

Main effects	Significance of F at				
	Week 4	Week 6	Week 8	Week 10	Week 12
Replicate	0.943 ns	0.714 ns	0.572 ns	0.349 ns	0.703 ns
Order	0.070 ns	0.023 *	0.137 ns	0.178 ns	0.623 ns

Note: ns = not significant at 0.05 level
* = significant at 0.05 level

If the experiment is analyzed as single block (without replication), Order 1 was found to be significantly different than Order 2 and Order3 at week 4 and week 6 only. Thereafter, no differences between the treatments (Table 3).

Table 3: Duncan ranking of order effect on rooting rate

Order	Duncan subsets for α=0.05				
	Week 4	Week 6	Week 8	Week 10	Week12
1	A	A	A	A	A
2	B	B	A	A	A
3	B	B	A	A	A

Note: Different letters within a column indicate significant different at α=0.05

Discussion

The percentage of rooted cuttings is higher (average 82% after 8 weeks) compared to two previous experiments:

1. Cutting from coppices of *K. ivorensis* (PISP Short note 11/10/91) by Yusrin Yusof & Laurent Hazard

   Rooting percentage after 8 weeks = 12%

2. Rooting rate of 0 generation (coppices) and 1st generation of *K. ivorensis* (August 1998) by Thomas Roling, A. Ramsah Mahalan & David Alloysius – paper in Bahasa Malaysia

   Rooting percentage after 8 weeks = 35% (0 generation)
   55% (1st generation)

The variation of rooting performances of *K. ivorensis* in these experiments could be explained with the following possibilities:

1. Cuttings from older trees are generally difficult to root. Clones showed a different coppicing and rooting ability (general observation)

2. *Khaya ivorensis* needs more time to roots than other species such as *Tectona grandis* and *Octomeles sumatrana*. Eight to twelve weeks are generally needed to produce acceptable rooting percentages.

3. The level of expertise in handling cuttings is increased within PISP staff

4. The current experiment was done in the newly renovated misting system near PISP office. This misting system is a closed-type with a standby generator to tolerate power disturbance if any. The new system undoubtedly provides more homogenous and conducive environment for production of cuttings.

Conclusions

Main conclusions derived from this experiment are:

1. The effect of order of cuttings on rooting is negligible. Cuttings of Order 1 root faster than Order 2 and Order 3, but final rooting percentage is not differed significantly after 6 weeks.

2. Cuttings start to root after 2 weeks on the sand bed. Eight to 12 weeks are needed to produce good rooting percentage (>80%).

Appendix 19

Kadar Pengakaran Keratan Generasi 0 dan Generasi 1 *Khaya ivorensis*

Oleh
Thomas Roling, A. Ramsah Mahalan, David Alloysius
Plant Improvement & Seed Production (PISP)
Ogos 1998

1. Pengenalan

Khaya ivorensis adalah salah satu spesies kayu bernilai tinggi yang ditanam di Pusat Perhutanan Luasong (PPL). Spesies yang berasal dari benua Afrika ini telah disenaraikan sebagai spesies kayu utama untuk aktiviti penanaman ICSB yang akan datang. Kaedah propagasi yang biasa untuk "African Mahogany" ini adalah melalui biji benih yang biasanya diimport secara langsung dari benua Afrika (Ivory Coast). Bagaimanapun pengimportan biji benih ini adalah sukar disebabkan jarak pemisahan yang terlalu jauh di samping lain-lain masalah seperti birokrasi, keadaan bekalan dan lain-lain. Untuk mengatasi masalah bekalan biji benih ini, maka kaedah propagasi secara pembiakan tampang dicadangkan untuk spesies ini. Kaedah pembiakan tampang yang biasa diguna adalah keratan batang (stem cutting).

Terdapat beberapa faktor yang mempengaruhi kejayaan penghasilan bahan tanaman melalui keratan batang, antaranya jenis klon, umur pokok induk, kaedah pengambilan dan sebagainya. Kebanyakan faktor-faktor ini belum pernah dikaji secara mendalam di PPL. Ini membuka ruangan untuk kajian pada masa-masa mendatang.

2. Tujuan

Percubaan ini dibuat dengan objektif-objektif yang berikut:

i. Menyelidik kadar pengakaran keratan (cutting) *K. ivorensis*
ii. Membanding kadar pengakaran keratan yang diambil terus dari pokok di lapangan (generasi 0) dan yang diambil dari pokok stok (generasi 1)

3. Kaedah

3.1 Sumber bahan

Kesemua bahan kajian adalah berasal dari percubaan provenansi (provenanace trial) yang ditanam di plot percubaan PISP. Percubaan provenansi ini (Plot KIV1) ini ditanam pada September 1990 dengan bahan-bahan tanaman dari tiga provenansi Afrika (Mopri, Yapo, Bonua) dan satu provenansi yang dikutip dari Kulim, Kedah. Satu aktiviti penjarangan (thinning) telah dilakukan pada Disember 1996 dalam percubaan ini yang telah mengurangkan jumlah pokok dalam plot dari 9 pokok (3x3 plot) kepada 5 pokok. Bahan-bahan kajian diambil dari batang pokok yang dijarangkan (tinggi tunggul >< 20cm). Terdapat sebanyak 32 tunggul yang dipilih sebagai sumber pengambilan keratan dan tunggul-tunggul ini ditanda sebagai klon KIV1, KIV2....KIV32. Tunggul-tunggul ini merupakan sumber untuk keratan generasi 0. Tidak semua tunggul ini aktif menghasilkan kopis. Keratan-keratan batang (single-node) diambil dari kopis-kopis yang sentiasa dicantas agar sentiasa muda (juvenile). Keratan-keratan yang telah berakar

dipindahkan ke pasu bersaiz 12" x 15" dan berfungsi sebagai pokok stok generasi 1. Apabila pokok stok generasi 1 ini telah cukup besar untuk menghasilkan keratan, maka percubaan ini pun dimulakan.

3.2 Penghasilan keratan

Keratan-keratan diambil dari kopis tunggul generasi 0 (G0) dan dari pokok stok generasi 1 (G1). Pengambilan keratan dibuat secara rambang tanpa menghiraukan identiti klon. Pemisahan hanya dibuat untuk mengasingkan keratan G0 dan keratan G1. Terdapat sejumlah 1027 keratan satu-nod (single-node cutting) diambil untuk setiap generasi (G0 dan G1).

3.3 Penjagaan dan rawatan terhadap keratan

Keratan-keratan yang diambil pada sebelah pagi direndam dalam larutan Thiram 80% (5g/liter) selama 30 minit. Hujung keratan kemudian dibubuh serbuk Seradix 3 dan dicucuk ke batas semai dengan media pasir yang telah dinyah-kuman. Sistem semburan dihidupkan dengan kadar semburan setiap 5 minit dan tempoh semburan 10 saat . Racun kulat dan racun serangga disembur bergilir-gilir dengan kekerapan setiap tiga hari.

3.4 Pemeriksaan pengakaran

Keratan-keratan diperiksa sebanyak 4 kali dalam tempoh dua bulan (hari ke-22, 30, 45 dan 60). Jumlah keratan yang berakar dicatat pada setiap pemeriksaan. Keratan yang mati dikeluarkan manakala yang masih hidup diletakkan kembali ke tempat asal.

4. Keputusan

Jumlah keratan yang berakar pada setiap masa pemeriksaan diringkaskan pada Jadual 1. Status keratan selepas 60 hari kajian berjalan dipersembahkan di Jadual 2.

Jadual 1. Jumlah & peratus keratan yang berakar setelah 22, 30, 45 dan 60 hari

Asal keratan	Hari ke-22	Hari ke-30	Hari ke-45	Hari ke-60
Generasi 0 (G0) 1027 keratan	72 (7.0%)	188 (18.3%)	277 (30.0%)	362 (35.3%)
Generasi 1 (G1) 1027 keratan	200 (19.5%)	376 (36.6%)	496 (48.3%)	564 (54.9%)

Jadual 2. Status keratan selepas 60 hari

Asal keratan	Berakar	Tidak berakar	Mati	Jumlah
Generasi 0 (G0)	362 (35.3%)	550 (53.5%)	115 (11.2%)	1027 (100%)
Generasi 1 (G1)	564 (54.9%)	400 (39.0%)	63 (6.1%)	1027 (100%)

Keratan-keratan yang telah berakar dipindahkan ke dalam pasu-pasu untuk penghasilan anak benih. Untuk keratan-keratan yang telah berakar, lebih kurang tiga bulan lagi diperlu untuk menghasilkan anak benih yang sedia untuk ditanam. Dengan ini adalah jelas bahawa penghasilan anak benih *K. ivorensis* dari keratan batang memerlukan masa sekurang-kurang 5 bulan dari tarikh pengambilan keratan.

5. Perbincangan dan kesimpulan

Seperti yang dinyatakan dalam kebanyakan kajian, pengakaran keratan adalah bergantung kepada status umur tanaman (juvenality) dari mana keratan itu berasal. Dalam percubaan ini jumlah dan kadar pengakaran untuk keratan dari G1 adalah lebih tinggi berbanding dengan keratan dari G0. Pokok-pokok stok G1 adalah berasal dari tunggul-tunggul di lapangan yang juga menghasilkan keratan-keratan G0 dalam percubaan ini. Dari segi fisiologi, 'umur' keratan-keratan dari G1 adalah lebih muda daripada keratan-keratan G0.

Kadar pengakaran untuk keratan-keratan G1 adalah cepat berbanding dengan keratan-keratan G0. Selepas tiga minggu kajian berjalan (hari ke-22), 19.5% daripada keratan G1 telah berakar berbanding dengan hanya 7.0% keratan G0 (Jadual 1). Jumlah pengakaran sebanyak 54.9% dari keratan G1 boleh dianggap baik terutama untuk *K. ivorensis* di mana belum banyak teknik penghasilan keratan yang diselidiki setakat ini.

Berdasarkan percubaan ini adalah dicadang agar penghasilan keratan batang untuk *K. ivorensis* dimulakan dengan penghasilan pokok-pokok stok di tapak semaian. Pokok-pokok stok ini lebih mudah diselenggara (pembajaan, penyiramana dll) berbanding dengan tunggul-tunggul di lapangan.

Appendix to PISP Report.

Summarized Activity Report of the Forest Regeneration and Research Unit (April 1998 - April 1999) for the ICSB / CIRAD-Foret Steering Committee Meeting No. 10

1. INTRODUCTION

This report is a summary of the activities carried out by the Forest Regeneration and Research Unit (FRR) in Taliwas, Lahad Datu. The mutual understanding among PISP, CIRAD-Foret and FRR is that all operational work in Taliwas i.e. maintenance of experimental plots are under the jurisdiction of FRR while processing of data collected for scientific writing-up and presentation will be carry out by PISP/CIRAD-Foret. As far as the ICSB / CIRAD-Foret Program is concern, FRR is covered under ICSB's PISP activity. Thus, extensive report on progress of the activity undertaken in particular of scientific papers and their dissemination shall be made available by PISP.

2. STAFF

To date staff status and their respective assignments are as follows :-

Designation	*No.*	*Assignment*
Conservation Officer	1	Unit in-Charge
Senior Forest Ranger	1	Nursery Operation
Forest Ranger	2	Nursery (Vegetative Propagation) / Field Operation
Casual Labourer (CL)	18	Nursery (9) / (9) Field Operation (km 13 / km 18)

The above list comprises of 3 PISP CL specifically looking after the experimental plots at Km 18, Taliwas. One RBJ Forest Labourer was transferred to Sandakan in July, 1998. In February,1999, 4 CL were re-employed from Luasong to assist the Unit in the operational production of teak.

3. OPERATIONAL WORK ON TRIAL PLOTS

Regular maintenance and monitoring work were carried out to the main experimental plots at Km 18 and Km 13, Taliwas. Specific observation or data collection is done while PISP and CIRAD-Foret carry out the scientific analysis. The overall operational activity carried out in 1998 for the respective trial plots significantly related to weeds control, thinning, site preparation for the establishment of new trial plots and other up - keeping and trees tending work.

4. TEAK PRODUCTION NURSERY

Sales made by the Unit within the reported duration were RM 89,404.40 in which some RM 63,600 were proceeds from sales of teak. This was represented by 21200 of teak cuttings / plantlets despatched for commercial purposes. Other income made from the nursery includes sales of cuttings or seedlings such as sentang, binuang, dipterocarps, laran while the rest topped up by sales of seeds.

A total of 2300 stockplants / hedgeplants maintained in 1998 and used for commercial production of teak shoots for macrocutting propagation. A total of 9675 cuttings were produced mainly in the second half of the year when most of the committed order was received from buyers. The commercial culling rate is 33% calculated from transferring of cuttings into misting beds until final delivery. In addition, a total of 24,473 plantlets were supplied by PBL. Out of this 16153 were successfully raised to their despatchable height.. Even though survivability rate during acclimatization was high, commercial culling reach up to an average of 34% (from the initial number received). Apart of mortality, commercial culling includes bad form material and overgrown plants.

Commercial despatched comprises of 8900 cuttings and 12,000 of plantlets. Balance of stock were used to replace older stockplants / hedgeplants (1 325), planted out (2600) and the rest culled as overgrown (1297). Commercial period allowable for teak under nursery condition is 6 months.

5. Trees Assessment

5.1 Tissue Culture Teak Plot

The actual Tissue Culture Teak plot comprises of 2 origins of teak propagated by macrocutting. All together 8 different origins was established using Randomized Complete Block design of 25 trees per unit plot replicated 4 times, planted in September, 1997 on a slope of 15 – 20 degrees at Km 13, Taliwas. It is considered as part of a demonstration plot but at the same time could be possibly use to determine performance of the 8 different origin of tissue culture trees taking Solomon Island's Clone 1 as control. The first measurement on height was taken 6 months after planting. However, it is still early to judge the result in term of best performing origin at this stage.

Table 1 : Mean Height of 8 Origins of Tissue Culture Teak Trees at 6 months old.

Origins	Mean Height (m)
Solomon Island (Bulk)	1.95
Solomon Island Clone 8	2.00
Ivory Coast (Bulk)	1.67
Perlis (Bulk)	1.90
Solomon Island Clone 9 ??	2.06
Ujung Padang (Bulk)	1.76
Macrocutting - Perlis (Bulk)	1.93
Macrocutting - Somon's Clone 1	2.19

Survivability within the recorded period is 96%. Mortality of 4% occurred equally to Ujung Padang (2 no.) and Perlis (Solomon Clone 9 ??) (2 no.). Die-back symptom was detected on Perlis Bulk (1%) and Perlis (Solomon Clone 9 ??) (2%).

5.2 Teak Stand Performance of Perlis seed Origin

A teak plot was established in late 1993 and was initially used to reforest the old aged cocoa areas. Presently they were maintained as part of a demonstration plot in relation to promoting commercialization of teak planting material produce from the nursery. However, the 4.3 years old (age

when assessment was made) teak stand came from a single origin of Perlis. Seed-bulk collection from candidate plus trees in Mata Ayer was arranged via FRIM, germinated in Taliwas and planted in December, 1993. Based on assessment made in the first part of 1998, the mean annual growth for height and diameter is 3.56 m and 4.58 cm respectively. Mean height and diameter at year 4.3 is 15.30 m and 19.70 cm, whilst height dominant achieved is 19.0 m and 25.0 cm for diameter.

6 Nursery Experiment

6.1 *Ex-vitro* Acclimatization Success of Acacias

The first batch of Acacias to be use for the Acacias Clones Test (*Acacia mangium* and hybrids) totaling of about 3000 plantlets comprising of various origins were sent from PBL for acclimatization at Taliwas nursery. Preliminary observation was made on acclimatization rate of success. Shoot rooted plantlets sent for acclimatization at Taliwas shows that within a time frame of 3 weeks under *ex-vitro* condition (under misting system), an achievement of 78.4% rooted plantlets was recorded. Survivability is 82.7%, 17.3% for mortality and 4.3% still not rooted.

In-vitro unrooted plantlet shows a much lower percentage rooting ability within the same time frame under ex-vitro condition. Number of rooted material achieved is 41.8%, 26.1% for no rooting development and 32.1% mortality occurrence. Acacia hybrids show an overall rooting performance 71.4% over 3 weeks in mist condition against 47.8% of *Acacia mangium*. Survivability for each of the Acacia group is 83.8% and 50.6% respectively.

Rooting performance within 3 weeks in *ex-vitro* acclimatization for all Acacias' treatment is 67.5%.

Table 2 : Overall performance of Acacia plantlets within 3 weeks in *ex-vitro* acclimatization.

Treatment Group	Root Condition *in-vitro*	Rooted (%)	Not Rooted (%)	Mortality (%)
A mangium	Shoot Rooted	53.5	0	46.5
	Shoot Not Rooted	28.1	12.4	59.4
A hybrids	Shoot Rooted	84.0	5.3	10.7
	Shoot Not Rooted	43.7	28.1	28.2

[Recommendation : Review supply of only *in vitro* rooted plantlet for *ex-vitro* acclimatization. These may ensure better survivability as well as high rooting success.]

6.2 Acclimatization Success of Teak Plantlet

Experiments were carried out to teak plantlets sent for acclimatization at Taliwas nursery to determine success rate of rooting in relation to their *in-vitro* shoot condition of plantlets before transferring to *ex-vitro* acclimatization i.e. root, callus formation. Some notes on result shown in Table 3.

Table 3 : Rooting Success Rate of Solomon Island plantlet within 6 weeks in *ex-vitro* acclimatization in relation to its shoot condition *in-vitro*.

Shoot Condition	Accumulated Percentage of Rooting Success Rate By Weeks					Accumulated Mor
in-vitro	2nd weeks	3rd weeks	4th weeks	5th weeks	6th weeks	in the 6th weeks
With **callus**, No root, No IBA	0	35	35	50	60	13
Without Callus, **With root**, No IBA	70	93	95	97	97	3
Callus Removed, No IBA	0	38	40	45	55	30
Callus Removed, With **IBA**	30	78	78	78	78	10
With **Callus**, With **Root**, No IBA	63	83	83	83	83	0

From the above, rooted planlet in *in-vitro* condition shows high percentage of rooting success within a shorter period when transferred in mist condition. Subsequently, certain percentage of plantlet shows no indication of rooting development within the 6 weeks period after transferring to misting beds. Prolonged observation to these plants shows lower percentage of rooting, averaging 41% after two months in the mist condition.

An experiment made to determine respond of cuttings taken from unrooted plantlets during *ex-vitro* condition shows that within 3 weeks, 64% rooted if apply with IBA and 55% without IBA treatment.

The notes above may be use as a guideline to supply the type of *in-vitro* plantlet for a better acclimatization success and effective production of teak for commercial purposes.

[Recommendation : 1. Culling of all plantlets when they do not root within 1 month or 6 weeks under *ex-vitro* acclimatization in mist condition. These will allow more space for 'good' plantlets production within acceptable time frame.

2. Review supply of only *in-vitro* rooted plantlet for ex-vitro acclimatization.]

Acacia hybrids

Items	Origins	Root Condition *In-Vitro*	Total Number of Plantlets	Mortality	Total Rooted	% Rooted
1	T3	R	44	-	35	79.5
		NR	63	6	34	53.7
2	2~28	R	84	4	77	91.7
		NR	29	14	10	34.5
3	5~15	R	37	10	19	51.4
		NR	72	3	64	88.9
4	5~13	R	34	2	27	79.4
		NR	86	28	30	34.9
5	6~15	R	50	-	50	100
		NR	54	5	34	63
6	4~15	R	130	-	124	95.38
		NR	2	-	2	100
7	AH~12	R	34	5	29	85.29
		NR	51	24	12	23.5
8	3~21	R	80	4	42	52.5
		NR	48	7	24	50
9	AH~16	R	46	4	42	91.3
		NR	34	1	-	18.8
10	10 LFC	R	56	5	47	83.9
		NR	14	3	-	-
11	11 LFC	R	19	1	18	94.7
		NR	62	13	28	45.2
12	3~19	R	74	25	41	55.4
		NR	48	31	9	18.8
13	7~23	R	42	7	35	83.3
		NR	72	33	39	54.17
14	6~23	R	121	11	110	91
		NR(n/a)				
15	8~19	R	88	7	81	92.1
		NR	11	5	3	27.3
16	10~24	R	94	6	88	93.6
		NR	3	3	-	
17	8~16	R	103	6	97	94.2
		NR	5	3	2	
18	6~5	R	76	3	73	96.1
		NR(n/a)				
19	AH~17	R	91	22	69	75.8
		NR(n/a)				
20	8~26	R	45	6	39	86.7
		NR(n/a)				
21	4~24	R	46	4	42	91.3
		NR(n/a)				
22	AH~14	R	17	1	16	94.1
		NR(n/a)				
23	AH~15	R	42	10	32	76.2
		NR	16	10	2	12.5
24	AH~11	R	8	8	-	
		NR(n/a)				
25	AH~10	R	6	6	-	
		NR(n/a)				

Acacia mangium

1	CL4	R	60	27	33	55
		NR	27	12	10	37.04
2	CL15	R	71	38	33	46.5
		NR(n/a)				
3	CL8	R	14	5	9	64.3
		NR(n/a)				
4	CL21	R	85	49	36	42.4
		NR	53	36	17	32.1
5	CL24	R	99	34	65	65.7
		NR	16	9	2	12.5

Legend : R = Rooted.
NR = Not Rooted.

AGENDA 4.2

PLANT BIOTECHNOLOGY LABORATORY (PBL)

PLANT BIOTECHNOLOGY UNIT

ACTIVITY REPORT

February 1998 – March 1999

ICSB

Dr. Doreen GOH
Ms. Hanna MOO
Mr. Japar MANUR
Mr. Hassan BASRI

CIRAD-Forêt

Dr. Antoine GALIANA

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FOREWORD

This report summarises the activities performed from February 1997 to March 1998 within the Plant Biotechnology Unit as an emanation of the CIRAD-Foret/ICSB ongoing collaboration.

1. INTRODUCTION - BACKGROUND OF THE PROJECT

The period April 1991 to July 1997 saw the inception and ongoing activities of the Plant Biotechnology Project, a collaborative effort between ICSB Forestry Division and CIRAD-Foret- known as CTFT at this time - signed a Supplementary Memorandum of Understanding - "SMOU" - for an In Vitro Culture Laboratory Project in Tawau (Sabah).

On February 15th, 1993, a second Supplementary Memorandum of Understanding was signed by both Parties in Kota Kinabalu to extend the duration of the project for 5 more years starting from July 10th, 1992, the date of signature of the Second Principal Memorandum of Understanding - "PMOU 2 "- in Paris.

With the ending of the PMOU 2 in July 1997, an extension of this collaboration was agreed between the two parties for a third PMOU. This PMOU3 was officially signed on September 21, 1998 for five more years and an evaluation of the collaboration in the third year. Proposal for several changes to the original Plans of Operation was made in this framework.

2. GENERAL ASPECTS RELATIVE TO THE PLANT BIOTECHNOLOGY UNIT

2.1. Objectives

The main objective of the common CIRAD-Foret/ICSB Plant Biotech laboratory has been to support Tree or Rattan Improvement and Planting programmes. Therefore, special attention has been devoted to the most rational way to utilise biotechnology to achieve this goal, being quite conscious of the respective limits of the conventional methods of tree improvement and vegetative propagation on one side and of the costs of tissue culture on the other as far as plant propagation is concerned.

The purpose of this laboratory was initially 100% Research & Development-oriented- to develop ICSB and CIRAD-Foret capabilities for direct transfer to the field or commercial transactions - consultancy, technology transfer, and sub-contracts. With the latter in mind, the new MOU comes with a proposal for commercialisation of *Tectona grandis* following the successful establishment of the protocol for mass multiplication of this species in the PBL over the past two years.

2.2. Relevant strategies

As described previously, two main fields of activities have been developed within the "Plant Biotech" unit to fulfil the objectives proposed, namely:

* Tissue culture, synonymous in our case with micropropagation;

* Electrophoresis investigations, with the use of biotechnological methodologies.

A detailed explanation of these two strategies had been given in the last two steering committee reports (1996-1997, 1997-1998). Interested parties are invited to refer to these reports for their further understanding of these strategies.

Through the application of the technique of tissue culture, the project was able to come up with workable protocols for the species studied. Consequently, mass multiplication of teak and Acacia species, of various clones and from different sources, has become possible. The most daunting task remains the dis-infection phase in which the success rate has not improved beyond 30% from each introduction. It is however important to note that the ability to introduce even a single explant for any clone would allow the possibility to further multiply this one clone under the specified tissue culture conditions. In other words, once the clone is successfully introduced without any contamination, the rejuvenation and subsequent multiplication of this clone become easier. Thus, as conducted on a routine basis, it became necessary to introduce as many explants as possible at different times of the year in order to ensure this possibility.

The conditions specified for routine tissue culture manipulations in the PBL are considered optimal in term of contamination problems (from bacterial and fungal sources) which have been minimised to a certain level. The occasional bouts of contamination that we observed usually arose partly as a result of the carelessness of the staff during manipulations of the plant materials or the failure to properly clean the glassware (test tubes or flasks). Other than this, contamination is usually of an endogenous origin and as such, is more difficult to control. The general up-keeping of the laboratory through the moping of the floor and cleaning of equipment on a regular basis definitely helped in the avoidance or reductions of such problems for an overall efficient running of the lab.

In the case of the use of electrophoresis for research, the work on rattans developed by Dr. Marie C. Bon has not been pursued since her departure. As decided in the previous steering committee meeting, the work on rattans would be considerably reduced in view of other activities such as commercialisation. This however does not mean that the technologies developed will not be put into use. On the contrary, with the availability of time and manpower, the technologies developed for rattans would be applied to the genetic analysis of teak and acacia for clonal/genotype identification or relatedness of certain individuals within or among populations. In relation to this, the six-week training of Hanna Moo on the Molecular Biology and Genetic analysis of Acacia

species and on *Rhizobium* species at the laboratory in December 1998 at Cirad-Foret, Baillarguet, Montpellier, is given in this report.

2.3. Equipment and staff resources

Basically, the CIRAD-Foret /ICSB Plant Biotech Laboratory has been conceived and planned to integrate research and development activities in complementary ways. The layout and the availability of the suitable equipment fulfil these two components, providing an ideal situation for the staff to carry out their work efficiently.

Information on the facility and available equipment has been detailed in the past Steering Committee Reports.

In addition to suitable electrophoresis equipment initially bought with French funds, new equipment have been purchased by ICSB to meet the increased activity in the lab, particularly for teak commercialisation. The most recent additions to the list are two units of beaker (2 Liter capacity) and a pH meter. The pH meter was necessary as the old unit which was obtained at the inception of the project was no longer operating optimally.

Again, it should be stressed that the PBL unit has been maintained in proper functioning conditions due primarily to the financial support of ICSB as far as the operational costs are concerned. This is of vital importance to the existence and upkeep of this project, and special attention has been devoted to reduce the running expenses as much as possible. During this reporting period, in the face of dealing with the economic woes resulting in numerous budget cuts, subscription to the two local newspapers was halted. As a result, the staff was obliged to look elsewhere for recycled papers in order to come up with the regular supply of transfer paper.

Other means of reducing the running cost of the laboratory are observed and followed as closely as possible. One of these is in the servicing of the generator set; instead of the monthly checking of the unit, this service was reduced to a bi-monthly routine, thus cutting back on the cost of maintenance.

The laboratory is now going into its sixth year of full operation. As highlighted in the last report, obvious signs of deterioration affecting the surface and other permanent indoor installations are now obvious. The most recent observation of the worsening condition is the obvious shifting of the ground level from the first floor, resulting in the enlargement of the crack in the ceiling between the two levels. This crack is definitely obvious in the structural column above the media preparation area. As a precaution, expensive and sensitive equipment such as the balances and magnetic stirrers were relocated to a different area. Close checking of this damage is in order on a regular basis. Another confirmation of this shifting of the ground is the inability of the doors leading to the tissue culture room and the washing area to be closed properly. It is most likely that this degradation is going to worsen as time passes.

Another serious and ongoing problem faced by the project is the cooling system in the tissue culture rooms. As a result of the constant breakdown of the air-conditioners in the first two culture rooms, specifically, the two rooms with 16:8 h lighting, a decision was made by the management to change the compressors of these two units instead of replacement with new units. These new compressors are now sustaining the system on a daily basis. It should be noted however that daily checking of the temperature has to be done to ensure that the fluctuations in the temperature regime are not too wide. It is now observed that the system is not operating at its most optimal and refilling of the cooling gas has to be done whenever required.

With the increased activity on both *Acacia* and teak in view of the service contract for SSSB and production for local teak planters, respectively, the third culture room which was kept in total darkness was once again converted to a 16:8 h light room in mid-1998. However, the lights were kept off for some of the shelves and which in turn were covered with black plastic sheets for any experiments requiring total darkness.

About staff, on ICSB side, the permanent personnel remains unchanged. These consist of:

Doreen Goh - Principal forest Officer/Co-project leader
Hanna Moo -Forest Officer,
Japar Manur - Assistant Forest Supervisor,
Hassan Basri, Iskandar Bahar, and Masdin Hamid - Laboratory Assistants
Sukmawati Bin Nurdin, - casual labourer,

and more recently, **Stephanie Ahning**, was recruited, also as casual labourer, to replace the previous student helper, Cham Lee Fong, who left for further studies in April after a stint of 4 months in the lab. The need for this new recruitment of CL was in view of the increased activity in the lab with the surge in local teak orders. The description of the duties of each of these staff was given in the last report; these duties have remained more or less unchanged. From Cirad-Foret side, **Dr. Antoine Galiana**, remains the co-project leader.

As in the past, a substantial part of the activities of the PBL has been devoted to staff training, to ensure a suitable handing-over and to gain in overall self-sufficiency of the staff in view of new activities such as in vitro and ex-vitro experiments for the species studied in the lab; the commercial production of Teak plantlets for sale, to overseas and particularly in this reporting period, the local markets; the multiplication of various teak origins and clones for field trials especially of the clones from Mata Ayer, Perlis based on the service agreement with FRIM; the introduction and multiplication of Acacia species particularly in view of the service contract with Sabah Softwoods Sdn. Bhd. with delivery of the requested amount of plants to occur early 1999.

The main outcomes from the research activities carried out by the PBL from February 1998 to March 1999 is reported hereinafter.

3. MICROPROPAGATION ACTIVITIES

3.1 Trees

3.1.1 Acacia species

Focus was placed on three species of *Acacia* during this reporting period, namely, *A. mangium, A. crassicarpa*, and *A. mangium X A. auriculiformis* hybrids. Several experiments were undertaken to determine the most optimal medium for rooting of *A. mangium* and *A. hybrids*. In addition, new introductions from the nursery and field were made for all three species for both research and in fulfilment of the service contract for Sabah Softwood Sdn. Bhd.

A. mangium

In addition to the two clones collected from mature trees of about 10 years old, that is, Clone No. 3 and 5, from the PNG seed stand in LFC, further attempts were made to introduce Clone No. 4 and 6. Past introductions had resulted in failure owing to the high level of bacterial contamination of both exogenous and endogenous origins. Finally, from two collections undertaken in June 1998, a single explant of only Clone No. 6 from each collection was successfully introduced and stabilised (Annex 2). This clone and the other two have been further multiplied and the current standing of each clone is as given in the table below:

Both clones 3 and 5, previously multiplied had been used in rooting experiments using different concentrations and types of rooting hormones such as NAA and IAA. However, the results obtained had not been very clear and as such, difficult to fully establish the most optimal medium. Most of the explants appeared to be unresponsive and over time, the absence of further growth prompted us to transfer these to fresh multiplication media without the addition of any growth regulators (GR). After three transfers to the GR-free basal MS medium, and in relation to the newly stabilised clone No. 6, all these three clones were placed on medium containing BA at 0.1 and 0.5 mg/l for further multiplication. Experiments using these clones will then be conducted, particularly on the aspect of rooting response, once there is a sufficient number of plant materials available.

Current standing of *Acacia mangium* clones in the PBL, from a 10-year old seed stand, PNG origin, LFC, since collections between June 1997 to March 1999

Clone Identifn	Date of introdn	No. explants Introd.	Total no. available
Am 3	June/July 1997	267	33
Am 4	August 1997	304	0
Am 5	August 1997	358	317
Am 6	June 1998	452	15

A. crassicarpa

The micropropagation of this species was first carried out on juvenile materials comprising of bulk seedlings; results from the preliminary studies were presented in the last steering committee report. The primary finding from these studies indicated that the medium for multiplication of *A. crassicarpa* is similar to that for *A. mangium* and rooting was spontaneous in all cases without the need for a rooting hormone. For more detailed information or results of these experiments, interested parties can refer to the last report.

In this reporting period, investigation on the responsiveness of mature materials collected from the field was made to determine if the rejuvenation of such materials was as promising as juvenile materials. Although an introduction of shoots from three 10-year old mature ortets from the LFC seed stand was made the previous year, the result was not reported. Out of these, only one clone (AC1) was successfully introduced. Having done this, it was decided to further collect shoots from other trees within the stand to further study this possibility. This effort was also made in view of the service contract for SSSB which list *A. crassicarpa* as one of the priority species for multiplication. Annex 1 shows the three to four years old SSSB clones collected at different dates from stock plants in the nursery and clonal plots in Brumas.

A selection of 9 clones was made from a seed stand of about 10 years old, PNG origin, from the Luasong Forestry Center (See Annex 2). These clones were placed under similar tissue culture conditions as those clones from SSSB which were collected a few weeks later (6 August, 1998). A comparative study between these two groups of plants based on the age of the ortets was made. A control consisting of bulked-up seedlings was also included in this study.

Based on preliminary results, the success rate of introduction from both batches of clones ranged from 0 to 30%, suggesting that the difference in maturity had no bearing on the introduction for in vitro culture. In other words, the rejuvenation of clones under the given tissue culture conditions and the medium used is not influenced by the physiological age of the ortets. In fact, within the same age group, the responsiveness varied according to the clone concerned, with clones such as AC1, AC 2, AC 11, AC 44 and PT 7 and PT 9 having a higher multiplication rate than the others.

In addition, these results have indicated that the medium for rooting is similar to that for multiplication without the need of any rooting hormone. Rooting appeared to be spontaneous once the plantlets were transferred from the multiplication medium consisting of a basal Murashige-Skoog medium with the proper concentration of cytokinin to one without any growth regulator. The data obtained so far showed a rooting rate ranging from 0 to more than 80%, again, depending on the clones involved for both batches of plant materials. For the control plants using seedlings, both multiplication and rooting response were as expected, very high, with rooting reaching 100% in all cases.

Table shows the responsiveness of clones under *in vitro* conditions from 4-year (SSSB clones) and 9-year (LFC clones) old *A. crassicarpa* plots

Clone Identity	Date Introd	No. explants introduced	% success of introduction	% rooting response	Present no. available
LFC Clones					
AC 1	10.8.97	48	2	83	122
AC 2	4.6.98	89	31.5	57	66
AC 3	23.7.98	191	22	16	17
AC 4	23.7.98	102	0	0	0
AC11	25.6.98	103	24.3	69	93
AC 29	25.6.98	36	0	0	0
AC 36	23.7.98	109	24.8	0	2
AC 37	4.6.98	150	4.7	28	6
AC 44	25.6.98	87	20.7	52	110
AC 45	23.7.98	149	29.5	12.5	24
SSSB Clones					
PT 1	6.8.98	48	8.3	80	9
PT 2	"	77	13.0	80	16
PT 3	"	98	2.0	0	3
PT 4	"	80	0	0	0
PT 5	"	80	17.5	42	12
PT 6	"	80	0	0	0
PT 7	"	103	62.5	53	93
PT 8	"	80	2.5	0	10
PT 9	"	80	30.0	25	37
PT 10	"	35	11.4	36	10
PT 11	"	48	16.7	60	27
PT 19	"	95	4.2	30	6

Note PT are the same clones as CR (refer to Annex)

This study will be pursued for two to three more cycles (6-8 week intervals) and data will be collected for these two parameters at each transfer. The medium used, although optimal for the study done so far, will be further tested particularly on such factors as the growth regulator and nutrient concentrations.

3.1.1.3. *Acacia mangium x auriculiformis* hybrids

Acacia species are generally propagated by seeds in large scale afforestation programs. However, this type of propagation can be sometimes limited due to the shortage of seeds or even good-quality seeds. In addition, a relatively high variability in the tree performances is sometimes observed between or within the progenies as in the case of *A. crassicarpa*. In the case of *A. mangium x auriculiformis* hybrids - that are known to have a better growth than the *A. mangium* pure parental species (Chia, 1993) - vegetative propagation is the only mean of propagation since propagation by seeds from bi-specific orchards still remains not feasible at the present time. The main interest of *Acacia* micropropagation is to rejuvenate and multiply mature selected plus trees. In *A. mangium* and *Acacia* hybrids, which are mature and generally exploited at 5 to 8 years-old, the negative maturation effects occur very early compared to many other tree species as attested by their early ages of flowering and fruiting that generally appear as soon as two years after germination. Considering the high cost of the tissue culture technologies, the genetic value of clonal materials in Acacias needs to be significantly higher than that of the best seed sources available before micropropagating clones.

So far, very few micropropagation studies or reports have been published on *Acacia* hybrids and none on *A. crassicarpa*. Conversely, micropropagation of *A. mangium* has been widely reported (Darus, 1991; Galiana, 1991; Bon *et al.*, 1998). Most of our experiments concerning the development of suitable micropropagation protocols and methods in *A. mangium* and *A. crassicarpa* were carried out in 1997 (see the last Steering Committee report, April 1998). Considering the importance of *Acacia* hybrids in the regional context and our current servive contract with Sabah Softwoods Sdn. Bhd., most of our experiments were focused on *Acacia* hybrids in 1998. However, some of the data obtained in *A. mangium* are reported hereafter for comparison with those obtained in *A. mangium x auriculiformis* hybrids. Our experiments on *Acacia hybrids* were mainly focused on the development of suitable protocols and methods for the three major micropropagation stages : *in vitro* introduction, multiplication and rooting. For the first time, *ex vitro* acclimatization experiments in nursery were also carried out on *Acacia* hybrids from the micropropagated materials. Lastly, owing to the development of a successfull micropropagation protocol in *Acacia* hybrids, about thirty clones from CIRAD-Forêt were micropropagated in the PBL for the setting up of a clonal test in Taliwas Forest Center.

A) - Plant material collection in the PBL

1) Sixteen different *Acacia* hybrid clones from CIRAD have been maintained in *in vitro* conditions since 1997 in the PBL after the transfer of their replicates from the CIRAD-Forêt tissue culture Laboratory (BSFT Laboratory in Nogent-sur-Marne) where they had been maintained since 1993. The numbers affected to these clones are as follows :

Clones no. 2-28, 3-19, 3-21, 3-26, 4-15, 4-24, 5-13, 5-15, 6-5', 6-15, 6-23, 7-23, 8-16, 8-19, 8-26 and 10-24.

These 16 clones originate from seeds collected in central Ivory Coast (Oumé). The seeds were collected in 1992 on five-year old *A. mangium* parent trees of unknown origin close to an *A. auriculiformis* plantation. Several plots established in 1990 at Oumé from seeds collected on the same original trees exhibited a quite high proportion of superior trees (about 10%) with all phenotypic characters of *A. mangium* x *auriculiformis* hybrids that are generally intermediate between *A. mangium* and *A. auriculiformis* pure parent species (flower colour, pod aspect, leaf shape and size, bark aspect and colour). From the one thousand seeds germinated in 1993 and grown for seven months in greenhouse (Nogent-sur-Marne, France), forty genotypes were selected. This early selection of the hybrids was based on two criteria : *i)* the putative hybrids were identified according to their specific morphological traits described by the Rufeld's identification method (Rufelds, 1987) and *ii)* their high nitrogen-fixing potential that was stimulated and expressed by growing the seedlings in jars on a nitrogen-free nutrient solution after they were inoculated with selected rhizobium strains. Recently, eight of these clones were genetically identified and confirmed by the Forest Genetic Laboratory of CIRAD-Forêt/Montpellier as *A. mangium x auriculiformis* hybrids through the use of two genetical identification methods: electrophoresis of isozymes and RAPD analysis (data obtained during Hannah MOO's training in the Forest Genetic Laboratory in CIRAD-Forêt Montpellier. France). The other eight clones were not analyzed.

2) Clones no. 8, 10, 11, 12, 14, 15, 16 and 17 that were introduced in Luasong nursery in 1994 originally came from the same seedlot as that of the 16 hybrid clones mentioned above but were not selected according to their nitrogen-fixing potential. All these clones except no.14 (not tested) were also genetically identified and confirmed as *A. mangium x auriculiformis* hybrids by the Forest Genetic Laboratory of CIRAD-Forêt/Montpellier.

3) Three other unselected clones from Luasong were also used for *in vitro* introduction experiments : clones A, B and C. These three ortets exhibited typical phenotypic traits of hybrids when collected (colour of flowers, leaf form, bark color), clone B being further genetically identified as an *A. mangium x auriculiformis* hybrid through isozyme and RAPD analyses (A and C not checked).

B) *In vitro* introduction of mature trees :

In order to test the cloning ability of adult trees through *in vitro* culture, the *in vitro* introduction stage was studied and performed on three *Acacia mangium x auriculiformis* hybrids and three *A. mangium* mature trees.

- Plant material and culture conditions :

The collection of *Acacia* hybrid shoots was performed on clones no. A, B and C (see their origins above in paragraph A-3). Three *A. mangium* ten-year-old genotypes from Taliwas Forestry Center were also used for *in vitro* introduction experiments : clones T2, T3, and T4 Clones T2 and T4 were identified as plus trees according to their growth performances and their oustanding bole straightness with no branches up to a minimum of 15 m height. The shoots were collected on low branches at 2 to 3 meters height from the ground for clones T3.

A, B and C or at the top of the trees for clones T2 and T4. The isolated shoots were divided into single nodal segments, disinfected with 0.25% (w/v) of mercuric chloride for 20 minutes before rinsing them in sterile distilled water and introducing into test tubes that contained the basal introduction medium for the primary culture. The tubes were placed under light with a daily photoperiod of 16 h in alternance with 8 h of darkness for two months at 28°C before observation and data collection.

- Results :

The data are reported in the table hereafter. This table shows that the percentage of contaminated plantlets obtained two months after transfer was higher in *Acacia* hybrids than in *A. mangium*, with a mean rate of about 72% and 45% respectively. The introduced shoots were mostly infected by exogenous or endogenous fungi with 89% and 84% of the total contaminations in *Acacia* hybrids and *A. mangium* respectively whereas the rest of the explants was contaminated by endogenous or exogenous bacteria. Although the contamination rate was quite high, especially in *Acacia* hybrids, all the genotypes tested were successfully introduced and propagated further with about 5 to 60% of survival according to the clone. Only a low proportion (0 to 15% according to the genotype) of contaminant-free explants did not exhibit any response whereas the successfully introduced explants responded positively by developing axillary shoots.

Introduction success and percentage of contaminations obtained two months after *in vitro* introduction of monodonal explants of *Acacia* hybrids and *A. mangium* collected from mature trees :

Clone no.	Species	Origin	Number of explants introduced	% of contamination		% of mortality	% of responsive explants
				fungal	bacterial		
T2	*mangium*	Taliwas	48	27.1	0	14.6	58.3
T3		"	85	45.9	20.0	14.1	20.0
T4		"	102	35.3	0	8.8	55.9
A	Hybrid	Luasong	48	89.6	2.1	4.2	4.1
B		"	38	52.6	5.3	0	42.1
C			34	58.8	20.6	2.9	17.7

- Discussion

According to other introduction data obtained on juvenile material, the age of ortet - at least in *Acacia* hybrids - does not seem to have any effect on the contamination rate and the reactivity of the introduced shoots. The results obtained in 1997 with shoots collected from 6-

month-old *Acacia* hybrid clones (clones no. 1, 8, 10, 11, 12, 14, 15, 16 and 17) showed high contaminations rates that reached 90% and 81% during two successive collections.

In terms of shoot reactivity, no major technical problem was encountered for *in vitro* introduction of *Acacia* shoots collected from mature trees, whatever the Acacia species concerned. Although quite high rate of infections were recorded during the introduction phase, especially in *Acacia* hybrids, microbial contaminations do not really represent a limiting factor since most of the contaminant-free nodal explants were reactive in producing elongated shoots from the axillary buds.

The level of rejuvenation and physiological age of this introduced mature materials are evaluated in the micropropagation experiments described in the following paragraph by comparing of their rooting ability with that of juvenile clones. One of the newly introduced mature clones from the experiment presented here (clone T3) has also been propagated and transferred to the nursery in Taliwas in view of field testing and in order to assess its rejuvenation level in comparison with juvenile or younger material.

C) Development of micropropagation media :

In Acacias, and in contrast with teak, the micropropagation process consists in transferring the plantlets on subsequent multiplication subcultures in a specific medium before to transfer all the multiplicated plantlets on a rooting medium at the end of the production cycle. The multiplication of shoots is obtained by stimulating budding, branching and elongation of the primary axillary shoots that are produced by the addition of growth regulators into the medium of culture. The elongated axillary shoots dissected and isolated from the shoot clusters obtained are then transferred onto new multiplication media.

As very few micropropagation studies and only very short notes have been published on *Acacia* hybrids so far (Darus *et al.*, 1992 ; Toda *et al.*, 1995), new experiments were needed to improve the multiplication and rooting rates by testing a larger range of medium component factors and culture conditions. Preliminary studies performed in CIRAD-Montpellier in 1995 showed that *Acacia* hybrids required a specific multiplication medium since the appropriate medium for *A. mangium* was not effective for hybrids.

Our studies were focused on the two micropropagation stages : multiplication and rooting. As the rooting ability of *Acacia* hybrids has never been tested, most of the detailed results reported hereafter are focused on the rooting stage.

- Culture conditions

The following experiments were performed in lighted culture rooms and in the same conditions under a daily photoperiod of 16 h in alternance with 8 h of darkness at 28°C. The explants were grown on a same basal medium including macro- and microelements, FeEDTA, vitamins and sucrose at a concentration of 2%. We used the same basal medium in both

multiplication and rooting media except that the strength in macroelements was diluted by two in the basal rooting medium. The clusters of axillary shoots obtained after the multiplication stage were dissected and the isolated shoots directly transferred to the rooting medium without any particular treatment except when specified. The plantlets were grown in tubes or flasks which contained 6 plantlets each in the multiplication experiments *versus* 10 plantlets per flask in rooting experiments.

- Plant material

In all the following multiplication and rooting experiments, three reference clones were used : no. 3-21, 5-13 and 7-23 (see the origins in paragraph 3-A above).

- Results and discussion :

1) Multiplication stage :

Many factors and medium components were tested in our multiplication experiments performed on the *Acacia* hybrids and more especially : different combinations of growth regulators, the strength of the medium in macroelements, different amino-acids and gelling agents. The table hereafter includes some of the main effects we observed on shoot multiplication of hybrids. In addition, the rooting percentage obtained after transferring the plantlets from the multiplication medium to the rooting medium was indicated in the same table as this percentage appeared to be correlated with the multiplication rate and then with the efficiency of the previous multiplication medium on shoot multiplication.

Three multiplication media containing different growth regulator combinations were tested The multiplication medium no.1 was the most efficient multiplication medium developed for *Acacia mangium* (see Steering committee report, 1998), multiplication medium no.2 was the optimal multiplication medium developed for *Acacia* hybrids and multiplication medium no.3 corresponded to the medium no.2 at a half concentration in growth regulators. Full strength and half strength in macroelements were tested for each of the three multiplication media.

The main shoot height and multiplication rate, that were used as multiplication indicators in the following experiment, were recorded at the end of 2 month-interval multiplication subcultures. Before this subculture, all plantlets were grown on the multiplication medium no.2 for at least three multiplication subcultures. The multiplication rate was calculated after transfer of the dissected shoots onto the new media. It was calculated according to the mean number of explants obtained per initial one after transfer of the dissected shoots onto new media. In the multiplication experiment, the data were recorded from 10 plantlets per treatment for clone no.7-23 and 5 plantlets per treatment for clones no.3-21 and no.5-13. In the subsequent rooting experiment, the plantlets originating from each multiplication medium after two months of growth were transferred onto a same basal rooting medium. The rooting

percentages collected from 10 to 40 plantlets per treatment were recorded two months after transfer.

Effect of different multiplication media on multiplication and rooting abilities of three *Acacia mangium x auriculiformis* clones * :

Multiplication medium no. **	Macroelement concentration in multiplication medium	Clone no.	Mean shoot height (mm)	Multiplication rate	Rooting %
1	Full strength	3-21	16.8	2.0	28.6
		5-13	9.0	2.6	50.0
		7-23	12.7	2.1	50.0
	Half strength	3-21	16.8	3.4	11.8
		5-13	8.6	3.4	36.4
		7-23	11.9	1.0	55.6
2	Full strength	3-21	8.2	1.8	55.6
		5-13	18.5	7.3	64.7
		7-23	15.3	4.0	70.0
	Half strength	3-21	11.0	1.2	0
		5-13	8.2	1.0	25.0
		7-23	11.3	2.2	54.5
3	Full strength	3-21	24.2	7.0	74.3
		5-13	15.3	2.5	71.4
		7-23	16.4	3.8	73.9
	Half strength	3-21	13.2	2.4	41.7
		5-13	14.8	1.8	42.9
		7-23	5.1	1.0	50.0

* Multiplication experiment : 10 plantlets per single treatment were tested for clone no.7-23 and 5 plantlets per treatment for clones no.3-21 and no.5-13.
Rooting experiment : 10 to 40 plantlets per single treatment were tested.

** - Multiplication medium no.1 corresponds to the multiplication medium usually used for *Acacia mangium* (see Steering committee report, 1998) ;
- Multiplication medium no.2 corresponds to the optimal multiplication medium developed for Acacia hybrids ;
- Multiplication medium no.3 corresponds to the medium no.2 at half concentration in growth regulators.

Mean multiplication rates and percentages of rooting obtained in the six different multiplication media, all clones combined :

Multiplication medium no. and strength	Multiplication rate	subsequent % of rooting
1 Full strength	2.23	42.9
1 Half strength	2.60	34.6
2 Full strength	4.37	63.4
2 Half strength	1.47	26.5
3 Full strength	4.43	73.2
3 Half strength	1.73	44.9

The table just above shows that the the medium composition in growth regulators (media no.1, 2 and 3) and its strength in macroelements had strong effects on shoot multiplication and the subsequent percentage of rooting. Medium 1, that was optimal for shoot multiplication in *A. mangium*, is not efficient for *Acacia* hybrids. With the two most efficient multiplication media no.2 and 3, a half strength in macroelements dramatically reduced the multiplication rate and even the subsequent percentage of rooting compared to the full strength. Media 2 and 3 that differed in growth regulator combinations had similar efficiencies on multiplication and rooting although a significant interaction was found between these two media and the different clones. Indeed, in multiplication medium no.2 at full strength, clone 5-13 had the highest multiplication rate (7.3) whereas clone 3-21 had the lowest one (1.8). Conversely, clone 5-13 had the lowest multiplication rate (2.5) and clone 3-21 had the highest one (7.0) in multiplication medium no.3 at full strength. By contrast, clone no.7-23 had a stable multiplication rate in both multiplication media no.2 and 3 with a mean multiplication rate of about 4.0. The mean shoot heigth is not always a good indicator for shoot multiplication since multi-stemmed clusters have necessarily more shorter shoots than mono-stemmed shoots which appear with low multiplication rates.

These data showed obviously that the best subsequent percentages of rooting were obtained with the explants originating from the most efficient multiplication media. Positive correlations were found between the multiplication rate and the percentage of rooting for each of the three clones tested (see the regression line and coefficient of determination obtained for clone 7-23 in Annex 9 p.82).

2) Rooting stage :

The main objective of these experiments was to improve the rooting percentage of *A. mangium* by testing different factors with a particular attention to the composition of the previous multiplication media. We analyzed the effect of different factors used in the previous multiplication subculture and/or in the rooting medium. All these rooting experiments were performed after several 2-month interval subcultures (at least two) on the optimal multiplication medium that was developed for *Acacia* hybrids (medium no.2 as described in the previous paragraph).

- Experiment 1 : Effect of different auxin concentrations on the rooting ability of three Acacia hybrid clones after2 months of culture on the previous multiplication medium

In this first experiment that was performed after two months of culture of the plantlets on multiplication medium, three auxin concentrations (0.2, 0.5 and 1.0 $mg.l^{-1}$) added to the rooting media were tested on the clones no. 3-21, 5-13 and 7-23. The data were collected three and seven weeks after transfer onto the rooting medium from 60 plantlets per treament. Each flask contained 10 explants. Then, the two-way analysis of variance was based on six replications per treatment corresponding to six flasks per treatment.

Percentages of rooting obtained 3 weeks and 7 weeks after transfer of the explants onto rooting medium according to the auxin concentration in the rooting media and the clone :

Clone no.	Auxin concentration ($mg.l^{-1}$)	% of rooting * after	
		3 weeks	7 weeks
3-21	0.2	20.3	48.0
	0.5	22.0	58.0
	1.0	32.4	64.7
5-13	0.2	8.1	19.9
	0.5	15.0	56.7
	1.0	18.3	39.1
7-23	0.2	16.3	58.0
	0.5	20.3	65.0
	1.0	18.3	52.5

* The rooting percentage was calculated from 60 plantlets per treatment.

Mean percentages of rooting according to each treatment, all other treatments combined :

Clone no.	% of rooting after 3 weeks	% of rooting after 7 weeks *
3-21	23.8	56.9 **b**
5-13	13.7	38.5 **a**
7-23	18.5	58.6 **b**

Auxin concentration ($mg.l^{-1}$)	% of rooting after 3 weeks	% of rooting after 7 weeks
0.2	14.9	40.6 **a**
0.5	18.9	60.0 **b**
1.0	21.7	51.4 **ab**

* The group letters attached to the percentages of rooting correspond to the homogeneous groups of means determined by the Newman & Keuls multiple range test at 5%.

The two-way analysis of variance (see the related statistical analysis in annex 10 p.83) showed that the two factors tested had significant effects on the percentages of rooting seven weeks after transfer of the explants onto the rooting medium (not calculated three weeks after transfer). No interaction between the two factors was found. The effect of clone was significant at P=0.01 and two homogeneous groups of means were identified according to the Newman and keuls multiple range test, clones no. 3-21 and 7-23 having a mean rooting percentage higher than that of clone 5-13 (see table below). The effect of auxin concentration in the rooting medium was significant at P=0.05, the best auxin concentration being the intermediate one (0.5 $mg.l^{-1}$).

- Experiment 2 : Effect of different auxin concentrations on the rooting ability of three Acacia hybrid clones after 6 weeks of culture on the previous multiplication medium

In this second experiment that was performed after six weeks of culture of the plantlets on the multiplication medium, two auxin concentrations (0.2 and 0.5 $mg.l^{-1}$) added to the rooting media were tested on the clones no. 3-21, 5-13 and 7-23. The percentage of rooting was observed one and two months after transfer onto the rooting medium from 60 plantlets per single treament. As in rooting experiment 1 above, each flask contained 10 explants. Then, the two-way analysis of variance was based on six replications per treatment corresponding to six flasks per treatment.

Percentages of rooting obtained 1 month and 2 months after transfer of the explants onto rooting medium according to the auxin concentration in the rooting media and the clone :

Clone no.	Auxin concentration ($mg.l^{-1}$)	% of rooting after *	
		1 month	2 months
3-21	0.2	25.0	43.3
	0.5	18.3	38.3
5-13	0.2	48.3	80.0
	0.5	20.0	61.7
7-23	0.2	21.7	48.3
	0.5	31.7	55.0

* The rooting percentage was calculated from 60 plantlets per treatment.

The two-way analysis of variance (see the related statistical analysis in annex 10 p.84) showed that only the clone factor had a significant effect (at P=0.01) on the percentage of rooting two months after transfer of the explants onto the rooting medium (not calculated 1 month after transfer) whereas the auxin concentration had no effect on rooting at P=0.05. Two homogeneous groups of means were identified according to the Newman and keuls multiple range test at 5%, clone no. 5-13 having a mean rooting percentage higher than that of clone 3-21 (see table below). Clone no. 7-23 was ranged in the two groups. No interaction between the two factors was found.

Mean percentages of rooting according to each treatment, all other treatments combined :

Clone no.	% of rooting after 1 month	% of rooting after 2 months
3-21	21.7	40.8 **a**
5-13	34.1	70.8 **b**
7-23	26.7	51.7 **ab**

* The group letters attached to the percentages of rooting correspond to the homogeneous groups of means determined by the Newman & Keuls multiple range test at 5%.

Auxin concentration ($mg.l^{-1}$)	% of rooting after 1 month	% of rooting after 2 months
0.2	31.7	57.2
0.5	23.3	51.7

We analyzed the same factors in experiments 1 and 2 except that the explants transferred onto rooting media were grown for two months on the previous multiplication medium in experiment 1 *versus* one and half months in experiment 2 (the plantlets tested in both experiments originated from the same multiplication medium). We found a clonal effect in both experiments whereas the auxin concentration had only a significant effect in experiment 1. However, only two concentrations of auxins were tested in experiment 2 against three in experiment 1. The rooting percentages were similar in both experiments as it reached a mean percentage of 51% in experiment 1 seven weeks after transfer onto rooting medium *versus* 55% in experiment 2 two months after transfer. The clones no. 3-21 and 7-23 had the same rooting percentages in both experiments. By contrast, clone no. 5-13 had the lowest rooting percentage in experiment 1 but the highest one in experiment 2.

- Experiment 3 : Effect of different types of auxins on the rooting ability of two types of shoots originating from two Acacia hybrid clones

In this third experiment, four auxin treatments including three different auxins added to the rooting medium (IAA at 0.3 $mg.l^{-1}$, IBA at 0.2 $mg.l^{-1}$ and NAA at 0.2 $mg.l^{-1}$) and a free-auxin control medium were tested on the clones no. 3-21 and 5-13. For each auxin treatment, two lengths of shoots were tested : *i)* entire axillary shoots with a length of about 4 cm and *ii)*

half cut axillary shoots including the top part with a length of about 2 cm. They were isolated from the shoot clusters at the end of the last multiplication cycle. The percentage of rooting was recorded seven and ten weeks after transfer onto the rooting medium from 20 plantlets per single treament. Each flask contained 10 explants. Then, the two-way analysis of variance was based on two replications per treatment corresponding to six flasks per treatment.

Percentages of rooting obtained from two Acacia hybrid clones 7 weeks and 10 weeks after transfer of the explants onto rooting medium according to the shoot lentgh and different types of auxin added to the rooting medium :

Clone no.	Shoot length	Type of auxin and concentration ($mg.l^{-1}$)	% of rooting * after	
			7 weeks	10 weeks
5-13	Long (entire shoot)	None	70	80
		IAA 0.3	40	80
		IBA 0.2	25	70
		NAA 0.2	35	75
	Short (half cut shoot)	None	50	70
		IAA 0.3	20	70
		IBA 0.2	0	25
		NAA 0.2	35	60
3-21	Long (entire shoot)	None	-	70
		NAA 0.2	-	80
	Short (half cut shoot)	None	-	100
		NAA 0.2	-	90

* The rooting percentage was calculated from 20 plantlets per treatment.

The two-way analysis of variance (see the related statistical analysis in annex 10 p.85) showed that the two factors tested had significant effects on the percentages of rooting of clone no. 5-13 seven weeks after transfer of the explants onto the rooting medium (data not analyzed statistically ten weeks after transfer since the experimental design was unbalanced due to fungal infections). No interaction between the two factors was found. The type of auxin had a significant effect at P=0.01 and two homogeneous groups of means were identified according to the Newman and keuls multiple range test : the plantlets grown on the auxin-free rooting medium had a mean rooting percentage about 2 times higher than that obtained on the media containing three different types of auxins (see table below). The effect of shoot length was significant at P=0.05, the long shoots having a mean rooting percentage about 60% higher than the short ones.

Mean percentages of rooting for clone no. 5-13 according to each treatment, all other treatments combined :

Auxin	% of rooting after 7 weeks	% of rooting after 10 weeks
None	60.0 **b**	75.0
IAA	30.0 **a**	75.0
IBA	12.5 **a**	47.5
NAA	35.0 **a**	65.0

Shoot length	% of rooting after 7 weeks	% of rooting after 10 weeks
Long	42.5 **b**	75.0
Short	26.2 **a**	56.3

* The group letters attached to the percentages of rooting correspond to the homogeneous groups of means determined by the Newman & Keuls multiple range test at 5%.

- Experiment 4 : Effect of different growth regulators used in the previous multiplication medium and in the rooting medium on the rooting ability of two Acacia hybrid clones

In this experiment, two auxin treatments (IAA at 0.3 $mg.l^{-1}$ and IBA at 0.2 $mg.l^{-1}$) applied to the rooting medium were tested on the clones no. 3-21 and 5-13. These auxin treatments were applied to axillary shoots originating from three different previous multiplication media that varied according to the hormone concentration (0.2, 0.3 and 0.4 $mg.l^{-1}$). The percentage of rooting was recorded 3 and 6 weeks after transfer onto the rooting medium from 12 plantlets per treament.

As the explants tested originated from three different multiplication media, they were separated in three distinct groups. However, this third factor was not further analyzed statistically since the mean percentages of rooting of each group were equal (see table just above). Then, a two-way analysis of variance was applied to the clone and auxin type factors. The statistical analysis calculated six weeks after transfer of the explants onto rooting medium (see in annex 10 p.86) showed that only the type of auxin added to the rooting medium had a significant effect on the percentage of rooting at P=0.05 (not calculated three weeks after transfer). The mean percentage of rooting obtained with IAA at 0.3 $mg.l^{-1}$ was 65% higher than that obtained with IBA at 0.2 $mg.l^{-1}$. No interaction between the two factors was found.

Although that was not significant, it was also found that IBA had a negative effect on rooting ability in the third rooting experiment described above since the rooting percentage was lower with IBA compared to the free-auxin control treatment.

Percentages of rooting obtained from two clones 3 weeks and 6 weeks after transfer of the explants onto rooting medium according to the hormone concentration in the previous multiplication medium and the concentration of auxin in the rooting medium :

Clone no.	Hormone concentration in the previous multiplication medium ($mg.l^{-1}$)	Type of auxin and concentration in the rooting medium ($mg.l^{-1}$)	% of rooting * after	
			3 weeks	6 weeks
3-21	0.2	IAA 0.3	25.0	50.0
		IBA 0.2	16.7	33.3
	0.3	IAA 0.3	16.7	33.3
		IBA 0.2	0	16.7
	0.4	IAA 0.3	16.7	50.0
		IBA 0.2	16.7	33.3
5-13	0.2	IAA 0.3	16.7	25.0
		IBA 0.2	0	16.7
	0.3	IAA 0.3	25.0	50.0
		IBA 0.2	0	25.0
	0.4	IAA 0.3	8.3	25.0
		IBA 0.2	16.7	16.7

* The rooting percentage was calculated from 12 explants per treatment.

Mean percentages of rooting according to each treatment, all other treatments combined :

Clone no.	% of rooting after 3 weeks	% of rooting after 6 weeks
3-21	15.3	36.1
5-13	11.1	26.4

Type of auxin in rooting medium	% of rooting after 3 weeks	% of rooting after 6 weeks
IAA 0.3 $mg.l^{-1}$	18.1	38.9 **b**
IBA 0.2 $mg.l^{-1}$	8.3	23.6 **a**

* The group letters attached to the percentages of rooting correspond to the homogeneous groups of means determined by the Newman & Keuls multiple range test at 5%

Hormone concentration in previous multiplication medium ($mg.l^{-1}$)	% of rooting after 3 weeks	% of rooting after 6 weeks
0.2	14.6	31.2
0.3	10.4	31.2
0.4	14.6	31.2

- Experiment 5 : Effect of different auxin concentrations and leaf trimming on the rooting ability of two Acacia hybrid clones

Three factors were tested in this experiment : the effects of leaf trimming (entire leaves and half cut leaves) and auxin concentration in the rooting medium (0.2, 0.5 $mg.l^{-1}$ and a free-auxin control treatment) were applied to the clones no. 3-21 and 7-23. The proportion of rooted shoots were recorded 2 and 5 weeks after transfer onto the rooting medium from 7 to 10 plantlets per single treament.

Number of rooted shoots obtained from two clones 2 weeks and 5 weeks after the second transfer of unrooted shoots onto rooting medium according to different concentrations of auxins and leaf trimming :

Clone no.	Leaf treatment	Auxin concentration ($mg.l^{-1}$)	Proportion of rooted shoots after *	
			2 weeks	5 weeks
3-21	Entire leaves	None	37.5	87.5
		0.2	50.0	-
		0.5	30.0	60.0
	Half cut leaves	None	30.0	40.0
		0.2	25.0	25.0
		0.5	40.0	70.0
7-23	Entire leaves	None	85.7	100.0
		0.2	28.6	42.9
		0.5	30.0	90.0
	Half cut leaves	None	57.1	85.7
		0.2	14.3	42.9
		0.5	0	0

* Rooting percentage was calculated from 7 to 10 replications per treatment.

The three-way analysis of variance did not show any significant effect of the three factors tested at P=0.05 (results not shown). However, as in the rooting experiment 3 described above, the best mean rooting percentage still remained the free-auxin rooting medium with 75% of explants rooted after five weeks *versus* 36.4% and 55% with auxin concentrations of 0.2 $mg.l^{-1}$ and 0.5 $mg.l^{-1}$ respectively. In the same way, the shoots with entire leaves had a rooting percentage after five weeks about 75% higher than those with half cut leaves although these percentages were not significantly different.

Mean percentages of rooting according to each treatment, all other treatments combined :

Clone no.	% of rooting after 2 weeks	% of rooting after 5 weeks
3-21	35.2	56.5
7-23	33.3	60.2

Auxin concentration ($mg.l^{-1}$)	% of rooting after 2 weeks	% of rooting after 5 weeks
None	50.0	75.0
0.2	30.0	36.4 *
0.5	25.0	55.0

* Missing value

Leaf treatment	% of rooting after 2 weeks	% of rooting after 5 weeks
Entire leaves	42.0	76.1
Half cut leaves	26.9	43.9

- Experiment 6 : Effect of a secondary transfer of unrooted shoots onto rooting medium

The unrooted shoots from clone no.5-13 in the rooting experiment 5 described above were transferred onto new rooting medium (secondary transfer) where two factors were analyzed : the effect of leaf trimming (entire leaves and half cut leaves) and addition of auxin in the rooting medium (0.2 $mg.l^{-1}$ and a free-auxin control treatment). The percentage of rooting was recorded 2 and 4 weeks after transfer onto the rooting medium from 30 plantlets per treament. Each flask contained 10 explants. Then, the two-way analysis of variance was based on three replications per treatment corresponding to three flasks per treatment.

Percentages of rooting obtained from clone 5-13 2 weeks and 4 weeks after the second transfer of unrooted shoots onto rooting medium according to leaf trimming and different concentrations of auxins :

Leaf treatment	Auxin concentration ($mg.l^{-1}$)	% of rooting * after	
		2 weeks	4 weeks
Entire leaves	None	46.7	54.1
	0.2	48.3	53.3
Half cut leaves	None	43.3	34.4
	0.2	31.0	40.0

* Rooting percentage was calculated from 30 replications per treatment.

The two-way analysis of variance did not show any significant effect on the two factors tested at P=0.05 (results not shown). However, as in the rooting experiment 5 described above, the shoots with entire leaves had a higher mean rooting percentage than the shoots with half cut leaves, 53.7% and 37.2% respectively after four weeks of culture, although these percentages were not significantly different. By contrast with the rooting experiments 3 and 5, the mean rooting percentages obtained in the free-auxin rooting medium and in the medium with an auxin at 0.2 $mg.l^{-1}$ were similar. This means that unrooted shoots after a secondary transfer onto rooting medium are less sensitive to auxins than the original shoots in the primary transfer.

- Experiment 7 : Effect of different concentrations of gelling agents and strength of rooting medium in macroelements on the rooting ability of Acacia hybrid clones

Two factors were tested in this experiment : the effect of two gelling agents at two different concentrations - Phytagel (Sigma) at 2.0 and 3.0 $g.l^{-1}$ and Gelrite Gellan Gum (Sigma) at 2.5 and 3.0 $mg.l^{-1}$ - and the strength of rooting medium in macroelements (half or full strength). The rooting percentage and the mean number of roots per plantlet were recorded 2 weeks after transfer of the axillary shoots onto rooting medium from 10 plantlets per single treament. In each treatment, the plantlets from clones no. 3-21, 5-13 and 7-23 were bulked together in equal proportions.

Percentages of rooting and number of roots obtained 2 weeks after transfer of the explants onto rooting medium according to the gelling agent and strength in macroelements :

Strength in macroelements	Gelling agent	Gelling agent concentration ($g.l^{-1}$)	Rooting % *	Mean number of roots/plantlet *
Half strength	Phytagel	2.0	20.0	0.4
		3.0	70.0	2.2
	Gerlite	2.5	80.0	1.5
		3.0	50.0	1.2
Full strength	Phytagel	2.0	30.0	0.5
		3.0	60.0	1.2
	Gelrite	2.5	40.0	0.5
		3.0	40.0	0.6

* Rooting percentage and mean number of roots per plantlet were calculated from 10 replications per treatment.

The two-way analysis of variance (see the related statistical analysis in annex 10 p.87) showed that the two factors tested had significant effects at P=0.05 on the number of roots per plantlet two weeks after transfer of the explants onto the rooting media. No interaction between the two factors was found. The gelling agent had a significant effect on the mean

number of roots per plantlet and two homogeneous groups of means were identified according to the Newman and Keuls multiple range test at 5% : Phytagel at 3.0 $g.l^{-1}$ was the best treatment with 70% to 280% more roots per plantlet than in the other treatments. On the other hand, the mean number of roots per plantlet obtained in the half strength medium was about two times higher than that obtained in the full strength medium. We obtained the same results with the rooting percentage that was shown to be correlated with the number of roots per plantlet.

Mean percentage of rooting and mean number of roots per plantlet according to each treatment, all other treatments combined :

Strength in macroelements	% of rooting	Number of roots/plantlets *
Half	55.0	1.33 **b**
Full	42.5	0.70 **a**

Gelling agent ($g.l^{-1}$)	% of rooting	Number of roots/plantlet *
Phytagel 2.0	25.0	0.45 **a**
Phytagel 3.0	65.0	1.70 **b**
Gelrite 2.5	60.0	1.00 **ab**
Gelrite 3.0	45.0	0.90 **ab**

* The group letters attached to the percentages of rooting correspond to the homogeneous groups of means determined by the Newman & Keuls multiple range test at 5%.

- ***Experiments 8, 9 and 10 :***

In these rooting experiments were analyzed the effect of different growth regulator combinations used in the previous multiplication medium on the rooting ability of different clones as well as the effect of activated charcoal in the rooting medium. The results were not detailed since no significant effect of this factor was found.

- Conclusion :

These experiments showed that *A. mangium x auriculiformis* hybrids can be quite easily micropropagated since their multiplication and rooting abilities are higher and less unpredictible than those of *A. mangium*.

The multiplication rates were highly variable according to the clone by contrast with the uniform multiplication rates obtained in *A. mangium*. Although only three *Acacia* hybrids clones were tested in the experiments described above, we have often observed this high variability among the other *Acacia* hybrid clones from our collection. As in the other *Acacia* species (see last Steering Committee report, 1998), the growth regulators have a determinant effect on shoot multiplication as well as other medium factors such as the gelling agent and strength in macroelements.

The rooting ability also depends mainly on the medium composition as it has been shown in the experiments above. The effect of auxin concentration was significant in all experiments except one. However, it appeared that the addition of auxins did not improved the percentage of rooting compared to the free-auxin control treatments. The gelling agent and medium strength in macroelements had also significant effects on rooting (percentage and number of roots) suggesting that the medium consistence (hardness) could have a physical indirect effect on root formation. The shoot aspect before transfer onto rooting medium was also important entire axillary shoots had a better rooting than half cut shoots. Leaf trimming by half induced a reduction of the rooting percentage when compared to the control treatment with intact entire leaves although it was not statistically significant. Although we rarely obtained 100% of rooting two months after transfer onto rooting medium in all the experiments described above, this maximal percentage was often observed three months after transfer eventually after a secondary transfer on the rooting medium. Another characteristic not reported in the data above was observed in Acacia hybrids : aerial roots emerging from the middle part of the shoot are often observed two to three months after transfer when no basal roots are produced one month after transfer onto rooting medium.

Clonal materials from other origins need to be tested to assess the rooting ability of Acacias hybrids in general. Actually, other clones from the same origin and from Sabah Softwoods were tested (see in the following paragraphs). Other factors seemed to have a stronger effect on rooting ability especially the physiological status of the axillary shoots at the end of the last multiplication cycle just before transfer onto rooting medium.

D) *Ex vitro* acclimatization of plantlets :

The acclimatization stage remains one of the main limiting factors for successfull micropropagation of tree species. After the rooting stage, plantlets must be transferred *ex vitro* to the misting system in the best conditions to reach a high survival rate, our goal in Acacias being to achieve more than 90% of survival like in teak. However, the absence of a basal callus in non-rooted Acacia shoots after 6 to 8 weeks of culture in rooting medium could affect the *ex vitro* acclimatization success, contrary to teak in which a large majority of non-rooted shoots that develop calli survives through the weaning stage. Due to this inability, the micropropagation success in *Acacia* species depends on a maximal *in vitro* rooting percentage before *ex vitro* transfer to the misting system.

The work presented hereafter was done in collaboration with Jikos GIDIMAN, Forest Officer in charge of the plantations and nursery in Taliwas Forest Center.

- Material and methods :

a) Transfer conditions in nursery

After two months of culture in the rooting medium, plantlets from the rooting experiments were brought to Taliwas nurseries and then taken out of the tubes. They were transplanted to sand beds (pure sand) under the misting system where shade was adjusted at

50 %. The frequences of misting were adjusted according to the standard conditions used in Taliwas for cuttings. After 1 month of acclimatization in sand, the plantlets were transferred to 1-liter polybags containing sandy-clay top soil. The plants in polybags were placed under the misting system for 2 weeks before final transfer under 50 % shade. The plants were watered twice a day for at least 2 weeks before reduction of the watering regime afterwards.

b) Plant material :

In this acclimatization experiment on *Acacia* hybrids, *A. mangium* was also tested as a control species. For each species, all the plantlets tested originated from the same *in vitro* rooting media (from the experiments reported in paragraph C-2 above). The plantlets were tested after different times of culture in the rooting medium.

- *Acacia mangium x auriculiformis* hybrids :

Three different *A. mangium x auriculiformis* hybrid clones were used in the following multiplication and rooting experiments : no. 3-21, 5-13 and 7-23.

- *A. mangium*

Three clones were used in our experiments : clones no.15, 21 and 24. Clones no.15 and 21 originated from three to five-year-old selected plus trees that were collected in 1989 in the south of the Ivory-Coast in the framework of a joint project between CIRAD-Forêt and IDEFOR/DFO (Institut des Forêts/Division Foresterie). After hedging and coppicing the trees, shoots from cuttings were introduced ***in vitro*** in the BSFT Laboratory (CIRAD-Forêt/Nogent-sur-Marne) from 1990 and maintained under multiplication until now. Clone no. 24 was an unselected clone originating from juvenile material (Rex Range provenance, Australia). It has also been maintained in *in vitro* conditions since 1989.

c) Data collection :

The survival rate of plantlets was recorded after one month of weaning under the misting system and two and half months, *i.e.* just after transfer to the polybags. The plantlets were separated into two distinct groups for both species : the pre-rooted plantlets that were already rooted before *ex vitro* transfer and the non-rooted ones.

- Results :

- Acclimatization of *Acacia mangium x auriculiformis* hybrids

As described in the table below, the overall percentage of survival obtained after four weeks of acclimatization, all clones combined, reached 94.4 % from 214 pre-rooted plantlets *versus* 75.9 % from 87 non-rooted ones. We did not observed any significant clonal effect on the survival rate whatever the age of plantlets when the treatments with a very low number of plantlets (n=4) were not taken into account.

Percentages of survival obtained in Taliwas during the acclimatization of *Acacia mangium x auriculiformis* hybrid *in vitro* plantlets four weeks after *ex vitro* transfer according to the clone, the presence of roots before transfer to the misting system and the age of the plantlets

Type of plantlets	Clone no.	Age of plantlets (months) *			Mean % per clone	Total mean %
		2	3	3.5		
Rooted	3-21	100.0 (16) **	-	94.4 (18)	**97.1** (34)	
plantlets	5-13	94.0 (67)	94.4 (72)	93.8 (16)	**94.2** (155)	**94.4** (214)
	7-23	-	-	92.0 (25)	**92.0** (25)	
Non-rooted	3-21	82.6 (23)	-	75.0 (8)	**80.6** (31)	
plantlets	5-13	79.7 (64)	0 (4)	92.3 (13)	**77.8** (81)	**75.9** (116)
	7-23	-	-	25.0 (4)	**25.0** (4)	
Mean from rooted plantlets for all clones combined		**95.2** (83)	**94.4** (72)	**93.2** (59)		
Mean from non- rooted plantlets for all clones combined		**80.5** (87)	**0** (4)	**76.0** (25)		

* The age of plantlets corresponds to the period of time starting from the last *in vitro* introduction of the plantlets into the rooting medium and ending at the moment of the *ex vitro* transfer under the mist system.
** Between brackets are indicated the total number of plantlets tested per treatment.

As shown in the table hereafter, the overall percentage of survival obtained after two and half months of acclimatization, all clones combined, dropped to 55.6 % from pre-rooted plantlets *versus* 38.8 % from non-rooted ones. As after four months, we did not observed any significant clonal effect on the survival rate. After two and half months of acclimatization, the age of plantlets seemed to have a significant effect on survival which was higher with 3.5-month-old plantlets than with 2-month-old plantlets, especially from non-rooted plantlets. Surprisingly, the lowest percentage of survival (only 19.4%) was obtained with three-month-old plantlets.

Percentages of survival obtained in Taliwas after the acclimatization of *Acacia mangium x auriculiformis* hybrid *in vitro* plantlets 2.5 months after *ex vitro* transfer according to the clone, the presence of roots before transfer to the misting system and the age of the plantlets

Type of plantlets	Clone no.	Age of plantlets (months) *			Mean % per clone	Total mean %
		2	3	3.5		
Rooted plantlets	3-21	62.5 (16) **	-	88.9 (18)	**76.5** (34)	
	5-13	73.1 (67)	19.4 (72)	81.2 (16)	**49.0** (155)	**55.6** (214)
	7-23	-	-	68.0 (25)	**68.0** (25)	
Non-rooted plantlets	3-21	26.1 (23)	-	62.5 (8)	**35.5** (31)	
	5-13	32.8 (64)	0 (4)	92.3 (13)	**40.7** (81)	**38.8** (116)
	7-23	-	-	25.0 (4)	**25.0** (4)	
Mean from rooted plantlets for all clones combined		**71.1** (83)	**19.4** (72)	**78.2** (59)		
Mean from non-rooted plantlets for all clones combined		**31.0** (87)	**0** (4)	**72.0** (25)		

* The age of plantlets corresponds to the period of time starting from the last *in vitro* introduction of the plantlets into the rooting medium and ending at the moment of the *ex vitro* transfer under the mist system.
** Between brackets are indicated the total number of plantlets tested per treatment.

- Acclimatization of *A. mangium :*

Percentages of survival obtained in Taliwas during the acclimatization of two-month-old *Acacia mangium in vitro* plantlets 4 weeks after *ex vitro* transfer according to the clone and the presence of roots before transfer to the misting system

Presence of roots before *ex vitro* transfer	Clone no.	Percentage of survival per clone	Total mean percentage
Rooted plantlets	15	62.9 (35) *	
	21	68.8 (96)	**65.4** (156)
	24	56.0 (25)	
Non-rooted plantlets	15	100.0 (3)	
	21	68.6 (51)	**59.1** (66)
	24	8.3 (12)	

* Between brackets are indicated the total number of plantlets tested per treatment.

On the three *A. mangium* clones tested, the overall mean percentage reached 65.4 % from 156 pre-rooted plantlets tested *versus* 59.1 % from 66 non-rooted ones. As in *Acacia* hybrids, no clonal effect on survival rate was observed.

- Conclusion

The acclimatization success obtained in *Acacia* hybrids was optimal one month after transfer to the misting system as it reached 95% of the pre-rooted plantlets and 75% of the non-rooted plantlets. By contrast, only two thirds of the pre-rooted plantlets were successfully acclimatized after one month in *A. mangium*. This could be explained by the different leaf morphologies of the two species. Indeed, the leaves produced by the three *Acacia* hybrid clones of these experiments are phyllodes whereas *A. mangium* always develops compound leaves with folioles which are less resistant to drying than phyllodes.

The percentage of survival obtained on *Acacia* hybrids two and half months after *ex vitro* transfer and then at the end of the acclimatization stage was not satisfactory. Other acclimatization experiments performed in Luasong Forestry Center gave the same survival rates whereas those carried out in Brumas in SSSB research nursery were more successfull since 95% of survival was recorded after three months of acclimatization.

E) FIELD EXPERIMENTS

Since the *Acacia* hybrid clones available from the CIRAD-Forêt collection and maintained in PBL have never been tested in the field, we decided to set up a clonal test including also some *A. mangium* selected clones and progenies as reference trees. The work presented hereafter was prepared in collaboration with Jikos GIDIMAN, Forest Officer in charge of the nursery and plantations in Taliwas Forest Center.

1) Preparation of the field trial :

- Plant material

Twenty-eight different *Acacia* clones from CIRAD-Forêt - including twenty-three *Acacia* hybrid clones and five *A. mangium* clones - and one bulk of selected *A. mangium* seeds were multiplied for further field assessment (see more information about origins of the *Acacia* clones in paragraph 3.1.1.3.A above)

- *A. mangium x auriculiformis* :

- Selected clones from CIRAD-Forêt : Clones no. 2-28, 3-19, 3-21, 4-15, 4-24, 5-13, 5-15, 6-5', 6-15, 6-23, 7-23, 8-16, 8-19, 8-26 and 10-24.
- Other clones from Luasong (same seedlot origin as clones no. 2-28 to 10-24) : Clones no AH8, AH 10, AH 11, AH 12 , AH14, AH 15, AH 16 and AH 17.

- *A. mangium* :

- Selected seeds from Luasong (bulk of progenies from the PNG seed orchard germinated and grown for two months in *in vitro* conditions) ;
- Selected plus trees from CIRAD-Forêt : Clones no. 4, 15 and 21 ;
- Unselected trees from Taliwas : Clone no. T3 (mature material tested as a control treatment) ;
- Unselected clone from CIRAD-Forêt : clone no. 24 (juvenile material from a Queensland origin)

Two batches were brougth to Taliwas for acclimatization : 2541 plantlets from 28 clones (plus 844 extra plantlets coming from multiplication media) on February 24, 1999 and 555 additional plantlets on March, 18 1999. Our objective was to produce 100 rooted plantlets per clone for acclimatization since 64 plantlets per clone were required for the experimental design The number of plantlets available for clones no. 4-24, AH8 and AH14 being not sufficient for field evaluation, they will bulked together to obtain 64 plants.

- Experimental design

The experimental design is a complete random block design including 4 complete blocks and at least 25 different clones and one *A. mangium* seedlot per block. Then, as a number of 16 trees per single plot has been choosed, a minimum number of 1644 plants will be necessary as planting material at the end of the weaning period. The plants will be probably transferred to the field next June. With a tree spacing of 3 x 3 m, this trial will cover a total area of about 1.5 ha and 1.8 ha including buffers.

2) Comparison of the *in vitro* rooting abilities of the different clones before transfer to nursery :

The rooting data were collected on the 21 *Acacia* hybrid clones micropropagated for the clonal test just before *ex vitro* transfer to the nursery. The proportions of phyllodes and compound leaves (folioles) produced by the different clones were recorded after two months of culture on a multiplication medium and just before transfer onto a rooting medium.

The table below shows that the proportions of phyllodes and compound leaves produced by the different *Acacia* hybrid clones have a very strong effect on their rooting ability since all clones producing at least 50% or a higher proportion of compound leaves (10 out of 21 clones tested) had an excellent rooting percentage ranging from 89 to 100% except clone no. 3-19 (total average= 94.0 %), only one month after transfer onto rooting medium. Conversely, the rooting percentage was significantly lower for all hybrid clones producing more than 50 % of phyllodes (11 out of 21 clones having 90 or 100 % of phyllodes) since it ranged from 23.5 to 74.3% (average = 52.8 %) two months after transfer onto rooting medium.

Percentages of *in vitro* rooting obtained 1 or 2 months after transfer onto rooting medium according to the origin and leaf morphology of different clonal materials :

Clone no.	*Acacia* species	Leaf composition *	Number of explants introduced	Number of rooted shoots	% of rooting after 1 mth	% of rooting after 2 mths
2-28	hybrid	90% Phyll. - 10% Fol.	113	84	-	74.3
3-19	"	100% Fol.	122	74	-	60.7
3-21	"	100% Phyll.	128	80	-	62.5
4-15	"	50% Phyll. - 50% Fol.	132	130	-	98.5
5-13	"	100% Phyll.	122	36	-	29.5
5-15	"	90% Phyll. - 10% Fol.	109	72	-	66.1
6-15	"	90% Phyll. - 10%	103	49	-	47.6
7-23	"	100% Phyll.	110	70	-	38.2
4-24	"	100% Fol.	46	46	100.0	-
6-5'	"	"	76	76	100.0	-
6-23	"	10% Phyll. - 90% Fol.	122	122	100.0	-
8-16	"	100% Fol.	107	102	95.3	-
8-19	"	100% Fol.	99	88	88.9	-
8-26	"	100% Fol.	45	45	100.0	-
10-24	"	40% Phyll. - 60% Fol.	97	94	96.9	
AH 10	"	100% Phyll.	81	56	-	69.1
AH 11	"	"	81	19	-	23.5
AH 12	"	"	85	35	-	40.0
AH 15	"	"	58	42	-	72.4
AH 16	"	"	80	46	-	57.5
AH 17	"	50% Phyll. - 50% Fol.	91	91	100.0	-

* % Phyll. = mean percentage of phyllodes per shoot ; % Fol. = mean percentage of compound leaves (folioles) per shoot ; % Phyll.-% Fol. = mean proportions of phyllodes and compound leaves on the same shoot.

General conclusion and perspectives

The micropropagation protocols developed in PBL on the three most important plantation *Acacia* species seem to be satisfactory, at least from the introduction to the rooting phases. The survival rate obtained at the end of the acclimatization phase needs to be improved. Since quite a number of medium components have been tested, the *in vitro* multiplication rate and rooting abilities of these species are almost optimal. Only the basal mineral composition of the multiplication medium requires additional experiments. The main limiting factor remains the bacterial contaminations as it can strongly affects the multiplication rate.

3.1.2. *Tectona grandis* (Teak)

Production of seedlings from in vitro germinated seeds

As reported last year, the seedlings from fourteen origins germinated *in vitro* were field-planted in a Progeny/Provenance trial in LFC as well as in Taliwas. These origins have been maintained at low numbers in the lab pending field information about their overall performance. These origins are:

Perlis,
Kota Marudu,
Bulu Kumba, Unjung Pandang,
Papua New Guinea,
Solomon Island I and II, and Solomon Island- Clones 1 to 9
India I, II, and III,
Thailand I and II
Ivory Coast

As reported, an origin trial was set up in Taliwas in October 1997 using microcuttings and macrocuttings from Unjung Pandang, Ivory Coast, Perlis, and Solomon Island. An assessment of the growth data of these plants will be done on a yearly basis.

Again, it should be noted that both Solomon Island bulk and Perlis origins have been multiplied intensively for use in commercial sales and several thousand plantlets had been sold during the past year to local buyers in the East Coast of Sabah. We are currently producing to fulfil the order of 34,000 plantlets from Kulumpang Development Company.

Several experiments have been conducted under in vitro and ex-vitro conditions using the two origins. A few clones of Solomon Island origin and Perlis origin-bulked material were also used in these experiments which will be described in the subsequent sections.

Mature plant materials

*** Microcuttings:**

As a routine task, there is still the emphasis to develop the staff capability for introducing nodal explants from mature plant materials with the best success rates. Based on the past and present introductions, the average percentage of success using the best treatment of 0.25% HgCl2 for 20 to 30 mins has not improved beyond 30%. Nonetheless, this level of success was good enough for our introduction purpose and finally, we managed to introduce and multiply all the 8 clones of Solomon Island origin (see Annex 4) plus Bulked-up materials. This was in relation to the recent successful introduction and

stabilisation of clone no. 6, which was the most problematic clone in term of bacterial contamination.

Collections of plant materials of Solomon Island origin were also made from the stock plants in Taliwas (see Annex 4). We observed that the success rate of plant material collected from the later and earliest part (Sept 1998 to early 1999) of the year was higher than at other time, reaching as high as 100% particularly for clones of Kota Marudu origin. It appears that the susceptibility of the stock plant could be influenced by the physiology of the plant itself at different growth stage or otherwise, the rate of infection and thus the spread of disease could be attributed to environmental factors such as spacing of the plants, humidity, and temperature. All these factors could contribute in one way or another to the success of the inoculation of the explants.

The individual clones which are rejuvenated and multiplied are maintained as such but would be eventually bulked up as new batches for the commercial production. This is necessary as the existing batches from previously introduced materials have been in the multiplication cycle for more than two years.

We observed that in batches that had undergone the normal cycle of multiplication which included an alternate addition or exclusion of growth regulator (GR) at every six to eight week- interval, the plants appeared to be in poor condition. These plants had large callus and are stunted in appearance, with more than one new shoot and an overall yellowish tinge. Following this observation, a decision was then made to eliminate the addition of growth regulator in the medium for the next several cycles. The development of the plants was closely followed during these cycles. After the third and fourth cycle, an obvious improvement in the appearance of the plants was seen. This was indicated by the colour, height, and absence of multiple axillary shoots which in turn result in the more vigorous growth of the plants.

These preliminary results indicated that even without any GR for several cycles (up to 4 months), the development of the plants was not hindered in any manner. This suggested that the exposure of plants to this GR for a prolonged period of time may not be necessarily conducive to further growth and thus, multiplication. On the contrary, the chemical might eventually have a limiting effect on the plantlets as it seemed to be in this case. In order to ascertain this result, we are continuing our observation on different batches of plants with the addition or omission of GR for different length of time. Such results will be noteworthy in view of mass production at an economical cost considering in particular, how costly these chemicals are. Equally more important will be the possibility to produce teak without the fear of genetic changes associated with the use of GR at any dosage over a long period of time.

In addition to the Solomon Island origin, we have also succeeded in multiplying clones from Kg. Apas and Kota Marudu (See Annex 4). Altogether, these consist of 12 clones as listed below:

Kampung Apas - 13, 16
Kota Marudu - 20, 22, 23, 25, 26, 32, 45, 47, 49, 51

About 150 plantlets from these two origins and Solomon Island were sent out to the LFC and Taliwas nursery for acclimatization at the end of February 1998. Of these, **Clones 3, 5, 7, 8, 16, 21, 22, 25, 41, and 42** including some clones from **meristematic** source were used in a methodology comparison trial with macro-cuttings of other clones as well as seedlings provided by PISP. This trial was reported as one of those planned in the 1998 Plans of Operation and was carried out in September 1998 on a 2 hectares plot next to the Progeny/Provenance Trial in Taliwas (Annex 5).

*** Shoot apical meristems:**

The introduction of shoot apical meristems of teak, ranging from 0.1 mm to 0.2 mm, on average, has allowed the introduction of true meristem cultures of mature teak genotypes. The advantages of introducing plant materials through the use of meristem were given in the previous report. Of the five mericlones 1, 3, 5, 7, and 8, introduced and maintained, we lost No. 7 which consisted of only one plantlet. After more than two years of maintenance under the given tissue culture conditions, the multiplication of these mericlones is in progress (see Annex 6). Among these four clones, mericlone 3 is the most vigorous in term of growth and multiplication rate. Clone 3 is also the only clone from which more than one meristems were successfully introduced, known in this case as mericlonal lines. It should also be noted that Clone 3 has been used extensively to meet teak orders although only a certain number of plants is allowed in each order met. This is to prevent a "monoclonal" effect in the event that this clone is observed to be more susceptible to any adverse environmental factors in the future.

Of the15 mericlonal lines obtained, only eight are multiplied further and kept at a minimal number of 30 for further observation and eventual use in further experiments. Eventually, once there is a sufficient number of mericlones 1, 5, and 8, in addition to 3 and its merclonal lines, a trial of these materials will be put out in the field. Results from such an intraclonal trial will be quite interesting for a more detailed assessment of its genetic value. Since the departure of Dr. Olivier in February 1997, no new introductions of meristems have been made.

Teak Experiments (*in Vitro and ex-vitro*)

During this reporting period, a few experiments under tissue culture conditions and in the misting nursery were initiated for teak using different origins and clonal materials. These experiments were undertaken to further understand and if necessary, optimise the conditions used for the mass multiplication and acclimatisation to ex-vitro conditions of the concerned species. Briefly, these experiments are summarised below

Experiment. I. – Survival of plantlets after storage for 1 to 7 days

This experiment was conducted to determine the survival of plantlets packed in plastic containers for a certain number of days. This is in view of commercialisation whereby packed plants will be transported abroad to cater for overseas market. Based on our recent experience in this activity in which 27,000 plantlets were sold to buyers in Peninsular Malaysia, about 95% of the plants sent was recorded to survive the acclimatisation phase under the given misting conditions. This result was based on after about 20 hours of confinement within the plastic containers in which they were placed, from the time of packing to their transfer onto the sand beds in the misting nursery the next day.

In order to further test the condition and survival of plants kept in the containers for a longer period of time, this experiment was undertaken using the following time frames: 1, 3, 5, and 7 days of confinement. These plants were packed inside the containers in the laminar flow hood and these boxes were in turn kept in carton boxes similar to those used for the transportation by air. After this treatment, the plants were then transferred to the sand beds in the misting nursery and observed. Survival of the plantlets was indicated by the general appearance of the plants as well as the growth of new roots at two and four week-interval followed by their transfer to polybags for the subsequent weaning and hardening phase.

Results of this study using plant materials consisting of Perlis-Bulk and Solomon Island Clone No. 3 are as given in the following pages.

Experiment II – Influence of callus on the rooting and survival of plantlets

This experiment was conducted to answer the question of whether the presence of callus on plantlets has any influence on the eventual rooting of these plants once exposed to *ex-vitro* conditions, i.e. during the acclimatisation in the sand beds. Previous observations suggested that such plantlets tend to remain alive for longer than four weeks but without the growth of new roots. This indicated that the callus on these plantlets may indirectly deter the production of new roots and in turn delay their transfer to polybags which may create a space constraint in the sand bed as more and more plantlets are transferred out for acclimatisation in the same period of time. In view of commercialisation prospect, this could become a limiting factor where space in the nursery is concerned. As such, there is a need to find out if manipulations such as removal of the callus and the addition of rooting hormone may increase the rooting response and thus, speed up the time of transfer to polybags.

Preliminary results taken from the study on plantlets from bulked-up material of Solomon Island origin are as given in the subsequent pages.

Experiment III- Influence of nutrients on growth of plantlets

This experiment was pursued for the past year to determine the effect of normal and half the concentrations of the macro- and micro-nutrients of the basal medium, with or without growth regulator, alternating between the two concentrations at every cycle of 4-6 weeks. The plant materials used are Clone No. 3 and 9 and progeny of 9, Solomon Island origin, and Perlis Bulk. The results from this study are as shown in the subsequent pages.

Experiment. IV.- Influence of iron and nutrients on growth of plantlets

This experiment was also pursued over the past year to determine the effect of normal and half the concentration of iron and nutrients in the basal medium for Clone No. 3, Solomon Island origin. Previously, plantlets from the origins Ivory Coast and Unjung Pandang were used in this study but the results were not very conclusive perhaps, owing to clonal effect within each origin as the plantlets were made up of bulked material consisting of several clones. As such, only a single clone was used to pursue this study. The results are as shown in the following pages.

Experiment I – Survival of plantlets after storage for 1 to 7 days

Plant material : Perlis and Solomon Island clone no.3

Objective : To compare the survival and rooting rate of plantlets stored in plastic boxes for seven days, five days, three days and one before acclimatization in the misting system. Data were collected three weeks after transfer.

Location : Taliwas Forest Center (3rd week data)

Plant material : PERLIS

	Day	7 days	5 days	3 days	1 day
Plantlets w/ roots w/o callus	survival %	94	100	97	90
	new root%	88	100	97	90
Plantlets w/ roots w/ callus	survival %	90	100	100	100
	new root%	40	70	88	88
Plantlets w/o roots w/ callus	survival %	100	100	--	100
	new root%	80	70	--	100
TOTAL	survival %	**93**	**1**	**95**	**93**
	new root%	**70**	**80**	**95**	**90**

Plant material : SOLOMON ISLAND CLONE NO.3

	Day	7 days	5 days	3 days	1 day
Plantlets w/ roots w/o callus	survival %	100	100	100	100
	new root%	80	83	100	100
Plantlets w/ roots w/ callus	survival %	92	92	100	100
	new root%	83	83	81	91
Plantlets w/o roots w/ callus	survival %	86	83	100	100
	new root%	29	67	67	80
TOTAL	survival %	**93**	**93**	**100**	**100**
	new root%	**69**	**80**	**83**	**93**

Location : Luasong Forest Center

Plant material : PERLIS (2nd week data)

	Day	7 days	5 days	3 days	1 day
Plantlets w/ roots w/o callus	survival %	100	100	100	100
	new root%	100	91	92	100
Plantlets w/ roots w/ callus	survival %	100	100	100	100
	new root%	29	75	69	50
Plantlets w/o roots w/ callus	survival %	100	100	100	90
	new root%	0	0	20	10
TOTAL	survival %	**100**	**100**	**100**	**97**
	new root%	**43**	**63**	**70**	**50**

Location : Luasong Forest Center

Plant material : PERLIS (4th week data)

	Day	7 days	5 days	3 days	1 day
Plantlets w/ roots w/o callus	survival %	100	100	100	100
	new root%	100	100	92	100
Plantlets w/ roots w/ callus	survival %	100	93	100	90
	new root%	71	47	77	80
Plantlets w/o roots w/ callus	survival %	80	100	100	90
	new root%	40	50	60	10
TOTAL	survival %	**97**	**97**	**100**	**93**
	new root%	**73**	**70**	**80**	**63**

Plant material : SOLOMON ISLAND CLONE NO.3 (2nd week data)

	Day	7 days	5 days	3 days	1 day
Plantlets w/ roots w/o callus	survival %	92	100	100	100
	new root%	77	55	92	67
Plantlets w/ roots w/ callus	survival %	100	100	100	100
	new root%	92	64	100	73
Plantlets w/o roots w/ callus	survival %	100	75	100	100
	new root%	83	25	40	70
TOTAL	survival %	**97**	**100**	**93**	**100**
	new root%	**84**	**50**	**87**	**70**

Plant material : SOLOMON ISLAND CLONE NO.3 (4th week data)

	Day	7 days	5 days	3 days	1 day
Plantlets w/ roots w/o callus	survival %	85	100	100	100
	new root%	85	100	100	78
Plantlets w/ roots w/ callus	survival %	92	100	100	100
	new root%	92	100	100	91
Plantlets w/o roots w/ callus	survival %	100	75	100	100
	new root%	100	75	80	90
TOTAL	survival %	**97**	**100**	**93**	**93**
	new root%	**90**	**93**	**97**	**87**

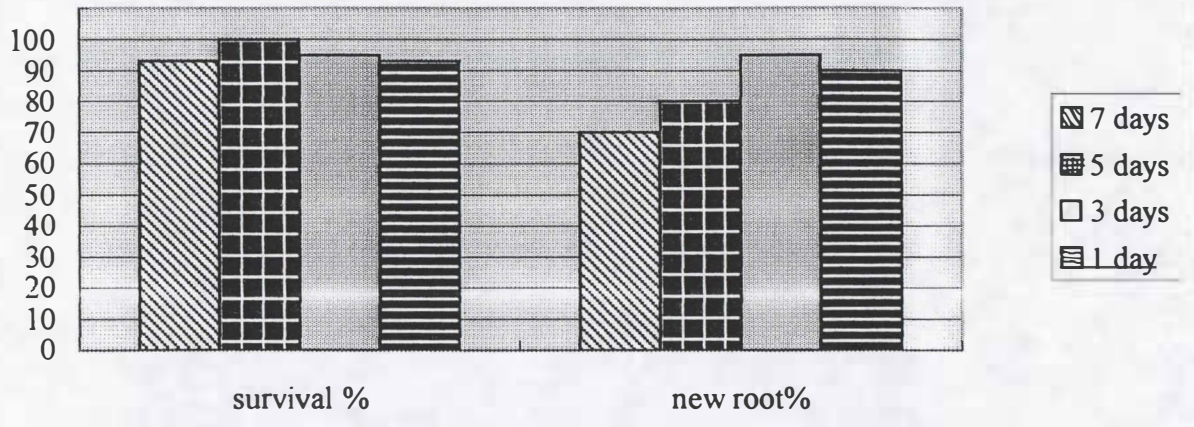
Tables and diagrams for the total of survival % and new roots % of each origin where the the experiments taken in different nursery.

Location : Taliwas Forest Center (3rd week data)

Plant material : PERLIS

Day	7 days	5 days	3 days	1 day
survival %	**93**	**100**	**95**	**93**
new root%	**70**	**80**	**95**	**90**

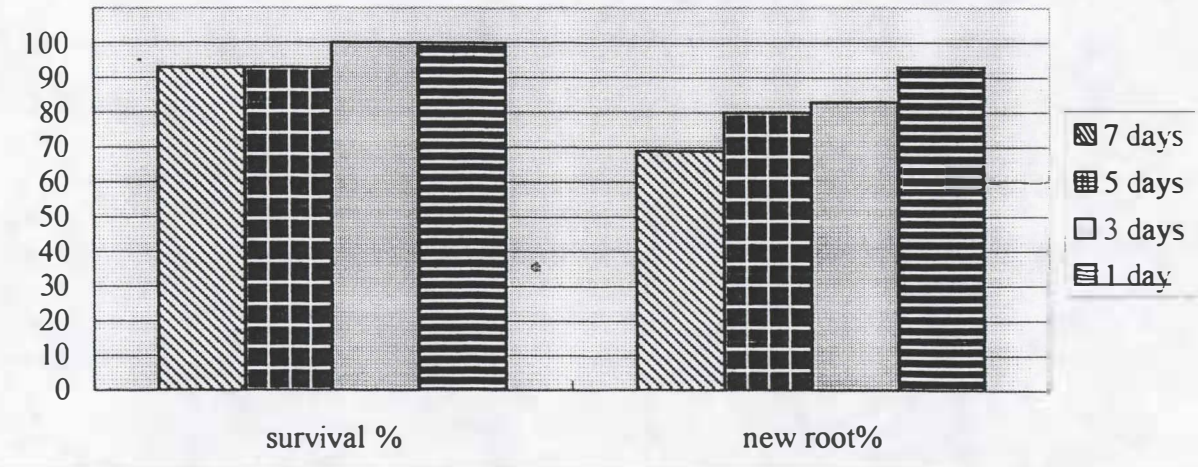
The survival rate and new roots development (%) of Perlis plantlets which have been stored in plastic containers for 7, 5, 3 & 1 day(s) before they were transplanted to the nursery.



Plant material : SOLOMON ISLAND CLONE NO.3

Day	7 days	5 days	3 days	1 day
survival %	**93**	**93**	**100**	**100**
new root%	**69**	**80**	**83**	**93**

The survival rate and new root development (%) of clone no.3 plantlets which have been stored in the plastic containers for 7, 5, 3 & 1 day(s) before they were transplanted to the nursery.

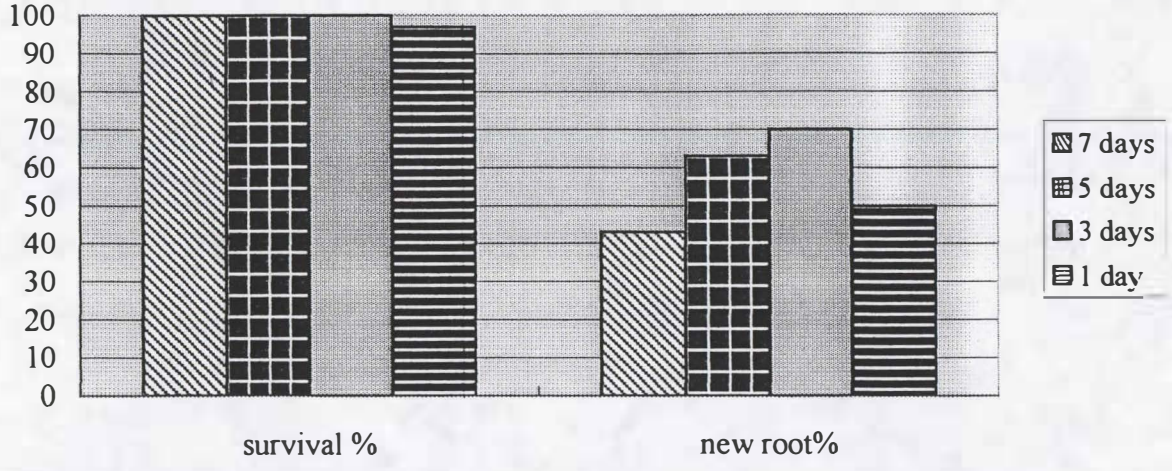


Location : Luasong Forest Center

Plant material : PERLIS (2nd week data)

Day	7 days	5 days	3 days	1 day
survival %	**100**	**100**	**100**	**97**
new root%	**43**	**63**	**70**	**50**

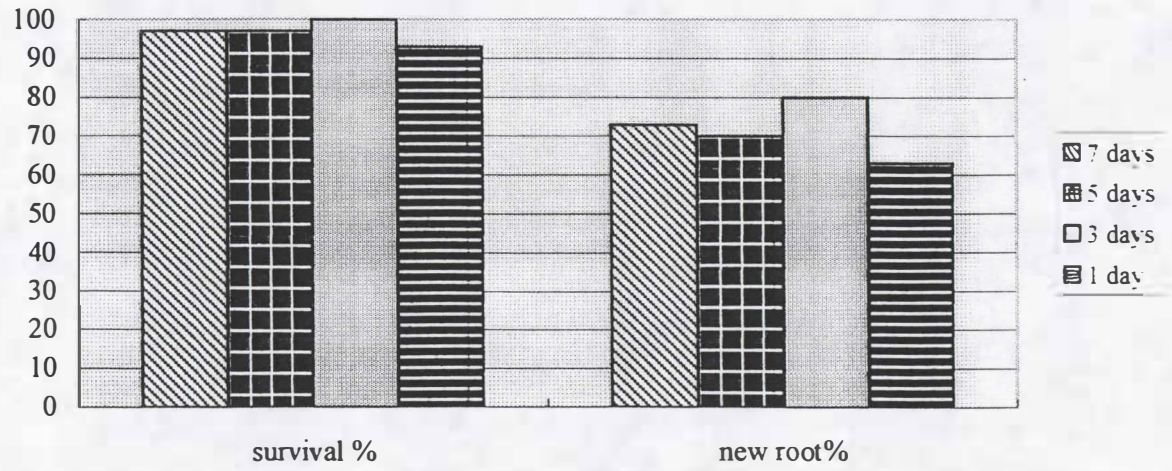
The survival rate and new root development(%)of Perlis plantlets which have been stored in the plastic containers for 7, 5, 3 & 1 day(s) before they were transplanted to the nursery



Plant material : PERLIS (4th week data)

Day	7 days	5 days	3 days	1 day
survival %	**97**	**97**	**100**	**93**
new root%	**73**	**70**	**80**	**63**

The survival rate and new roots development(%)of Perlis plantlets which have been stored in the plastic box for 7, 5, 3 & 1 days before they were transplated to the nursery.

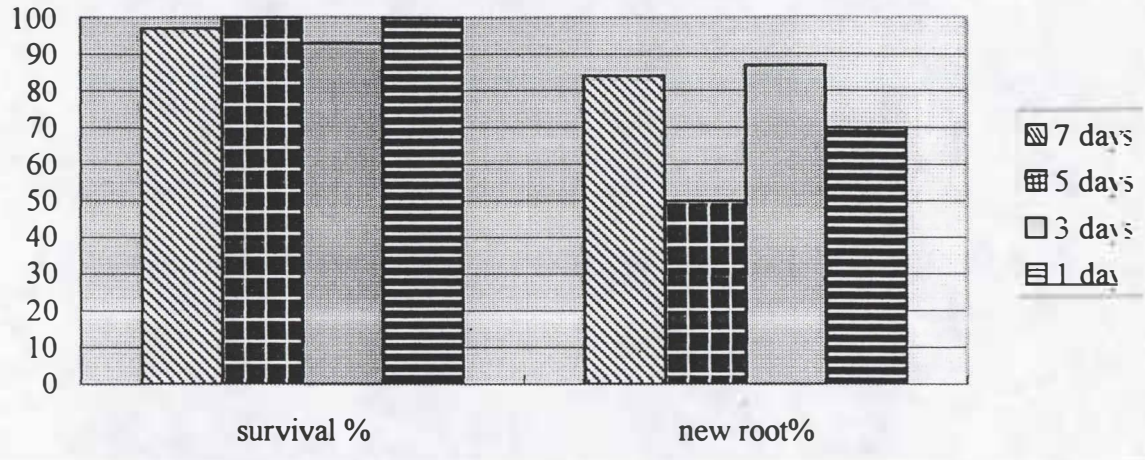


Location : Luasong Forest Center

Plant material : SOLOMON ISLAND CLONE NO.3 (2nd week data)

Day	7 days	5 days	3 days	1 day
survival %	**97**	**100**	**93**	**100**
new root%	**84**	**50**	**87**	**70**

The survival rate and new roots development(%)of clone no.3 plantlets which have been stored in the plastic boxes for 7, 5, 3 & 1 day(s) before they were transplanted to the nursery.

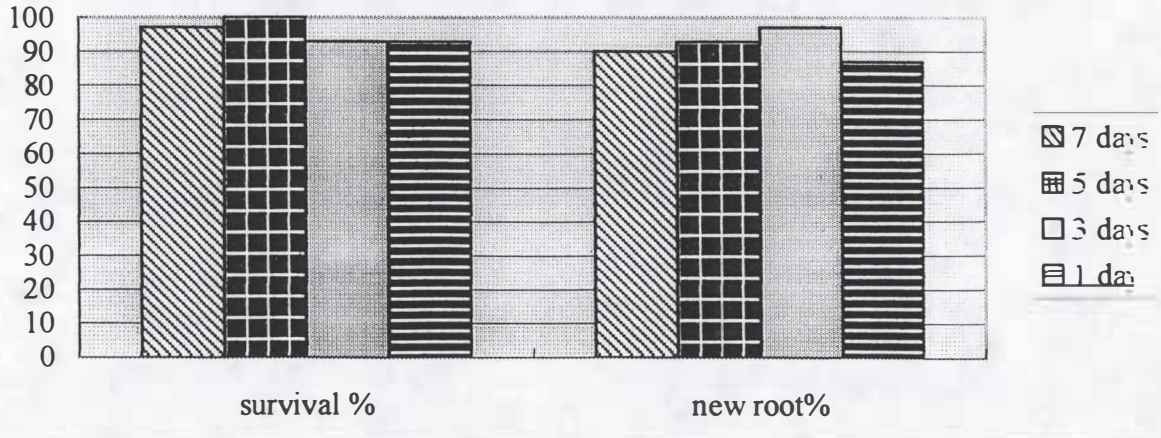


(4th week data)

Plant material : SOLOMON ISLAND CLONE NO.3

Day	7 days	5 days	3 days	1 day
survival %	**97**	**100**	**93**	**93**
new root%	**90**	**93**	**97**	**87**

The survival rate and new root development(%)of clone no.3 plantlets which have been stored in the plastic containers before they were transplanted to the nursery.



Results: (1) No significant difference could be observed among the time periods of storage; the average survival was more than 90% in all cases.

(2) The presence of callus appeared to influence the rooting response of plant only if the plantlets did not have any roots at the time of transfer. This result is however not so clear for Perlis as this origin consists of bulked materials.

(3) There is no direct correlation between percentage of new roots and percentage of survival i.e the survival of plantlet was not necessarily due to the production of new roots.

Conclusion: This study established that plantlets could be stored for up to 7 days in plastic boxes without any adverse effect on survival of plantlet following acclimatization. This result is useful when there is a need to transfer plantlets to distant markets requiring such time of storage. This brightens up the prospect for overseas markets.

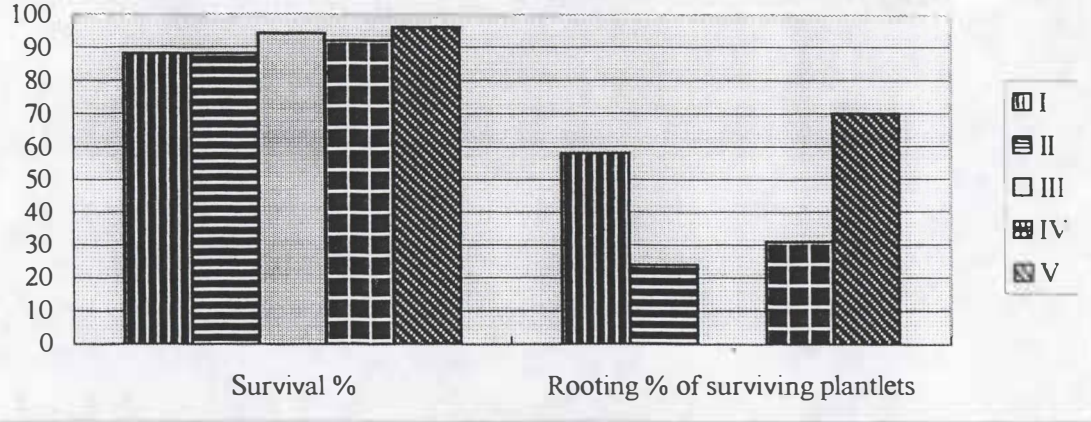
Experiment II -- Influence of callus on the rooting and survival of plantlets

Plant material : Solomon Island Bulk & Solomon Island Clone no.3 (data for clone no.3 not yet collected)
Objective : To determine the rooting response of plantlets following the treatment below
Treatment : (I) Whole plant, rooted with callus but the calllus was removed, with Seradix II
(II) Whole plant, rooted with callus but the callus was removed, without Seradix II
(III) Whole plant, not rooted with callus, without Seradix II
(IV) Whole plant, rooted with callus, without Seradix II
(V) Whole plant, rooted without callus, without Seradix

Plant material: Solomon Island Bulk (2nd week data)

Treatment	I	II	III	IV	V
Number of plantlets	52	51	51	48	50
Survival %	88	88	94	92	96
Rooting%of suv.plt	58	24	0	31	70

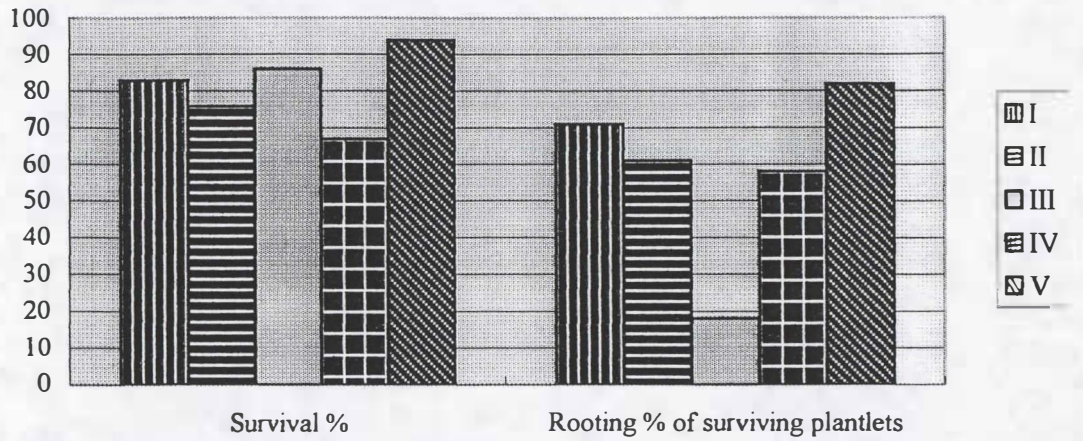
The survival rate and new roots development(%)of Solomon Island bulk plantlets which were under 5 different treatments on the misting bed.



(4th week data)

Treatment	I	II	III	IV	V
Number of plantlets	52	51	51	48	50
Survival %	83	76	86	67	94
Rooting%of suv.plt	71	61	18	58	82

The survival rate and new roots development(%)of the Solomon Island bulk plantlets which were under five different treatments on the misting bed.



Results: (1) For whole plant which are rooted but with or without callus, treatment with Seradix is not necessary.

(2) For whole plant which are not rooted but with callus, removal of the callus with the addition of Seradix improved the rooting rate. This is based on observation that the plantlets with callus did not root as quick ly as those without.

(3) Although no new roots are produced on the plantlets, they remain healthy for longer than 4 weeks. This could be a constraint as the plants would not be transferred to polybags without any roots; such plants tend to die after transfer.

(4) There is no direct correlation between the survival rate and the rate of rooting of the plantlets.

Conclusion: This study established that plantlets which are already rooted did not require any growth regulators. It also showed that the presence of callus did influence the production of new roots from the plantlets. Although these plantlets did not root they remain alive, suggesting that callus may be sustaining the plantlets. If the callus was removed and rooting hormone was used, new roots were produced more quickily.

Experiment III -- Influence of nutrients on growth of plantlets

Plant material: Solomon Island clone no.3, Solomon Island clone no.9 (MBO), progeny clone no.9 (MDESC) and Perlis. The experiment has been conducted for more than 1 year consisting of transfer at 6-week intervals.

Objective: To determine the concentration of the nutrients influence the growth rate of the plantlets.

Treatment : (I) Normal cycle with full concentration of nutrients, with or without BA.

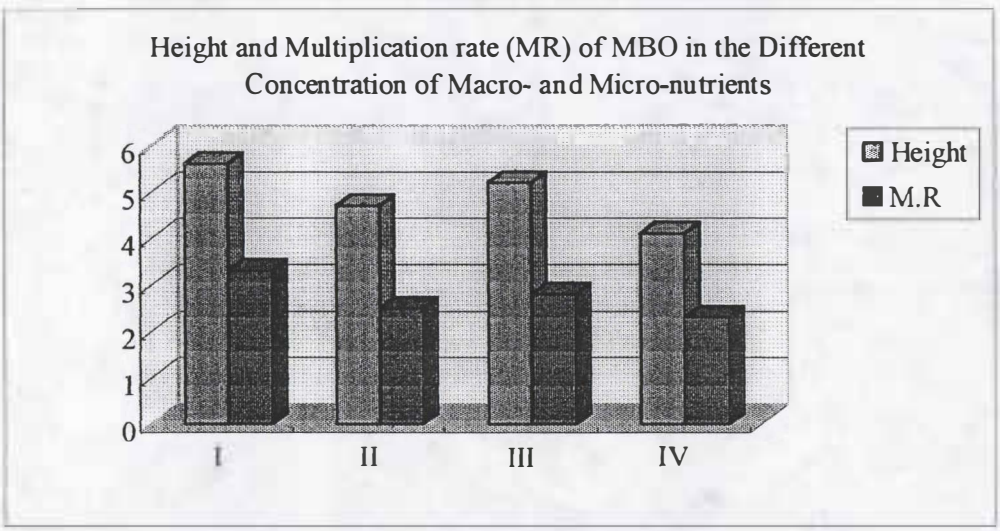
(II) Alternate cycle with full concentration of nutrients without BA, half concentration of nutrient with BA.

(III) Alternate cycle with half concentration of nutrients without BA, full concentration of nutrient with BA.

(IV) Normal cycle with half concentration of nutrients, with or without BA.

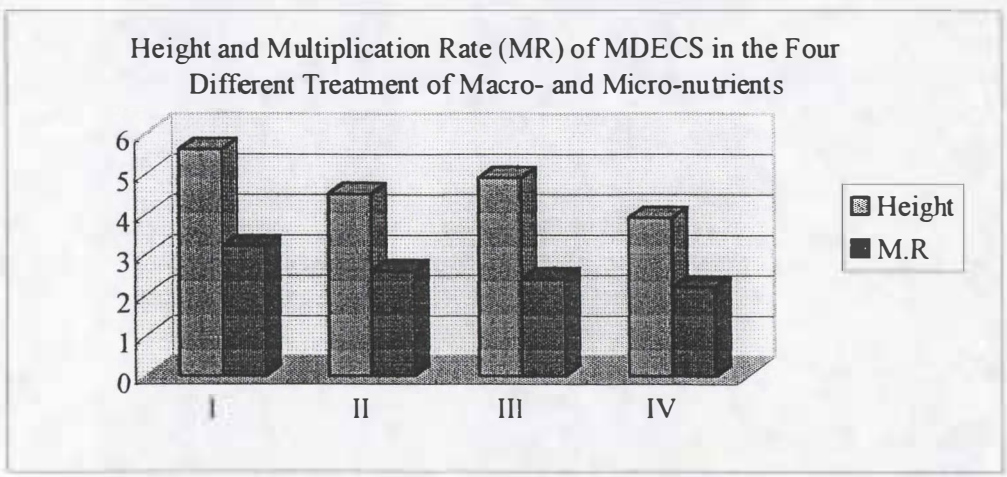
Plant material: MBO

Treatment	I	II	III	IV
Height	5.6	4.7	5.2	4.1
M.R	3.3	2.5	2.8	2.3



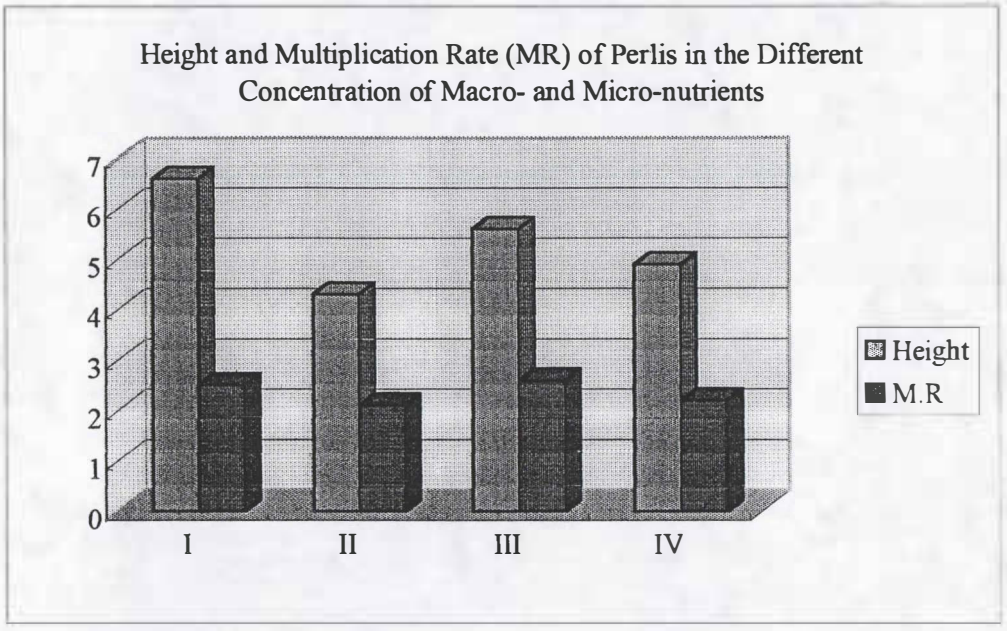
Plant material: Progeny clone no.9 (MDESC)

Treatment	I	II	III	IV
Height	5.6	4.5	4.9	3.9
M.R	3.2	2.6	2.4	2.2



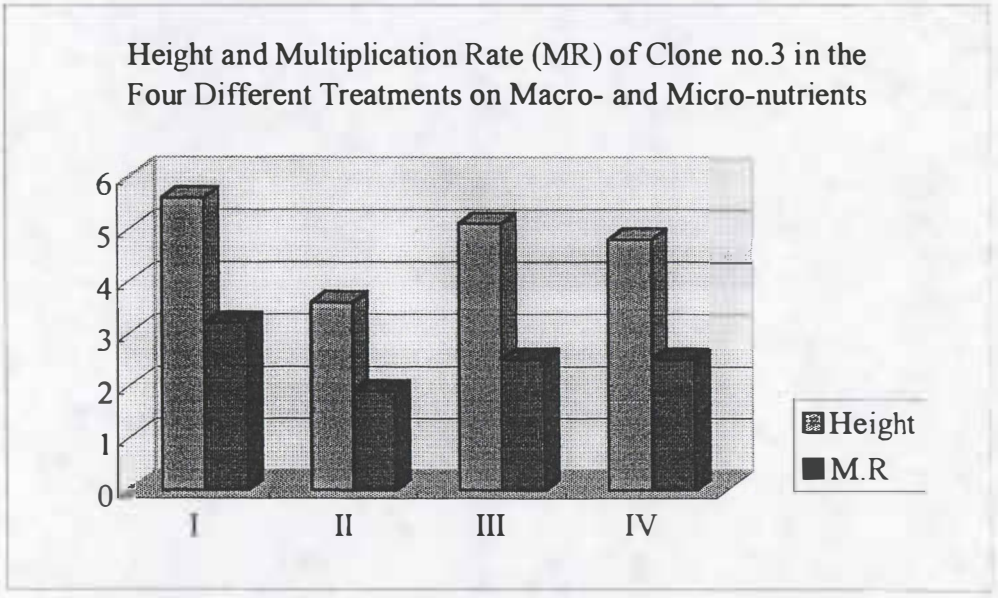
Plant material: Perlis

Treatment	I	II	III	IV
Height	6.6	4.3	5.6	4.9
M.R	2.5	2.1	2.6	2.2



Plant material: Solomon Island Clone no.3

Treatment	I	II	III	IV
Height	5.6	3.6	5.1	4.8
M.R	3.2	1.9	2.5	2.5



Results: (1) The result showed that the plants maintained on normal cycle with full concentration of nutrients and those on alternate cycle with half concentration of nutrients without BA and full concentration of nutrients with BA media, did have a higher multiplication rate. The result is not similar for progeny clone no.9(MDESC) and Perlis even though these origins consist of bulked materials except the difference is not as significant.

(2) There is a direct correlation between the height and multiplication rate i.e the higher plantlets will give higher multiplication rate. Again, it is not so clear for progeny clone no.9 (MDESC) and Perlis as these origins consist of bulked materials.

Conclusion: This study established that using medium with normal concentration of nutrients with BA and alternated with medium without BA gave the best multiplication rate. However, this consists of half concentration of nutrients without BA alternated with concentration with BA was quite optimal.

Experiment IV – Influence of Iron and nutrients on growth of plantlets

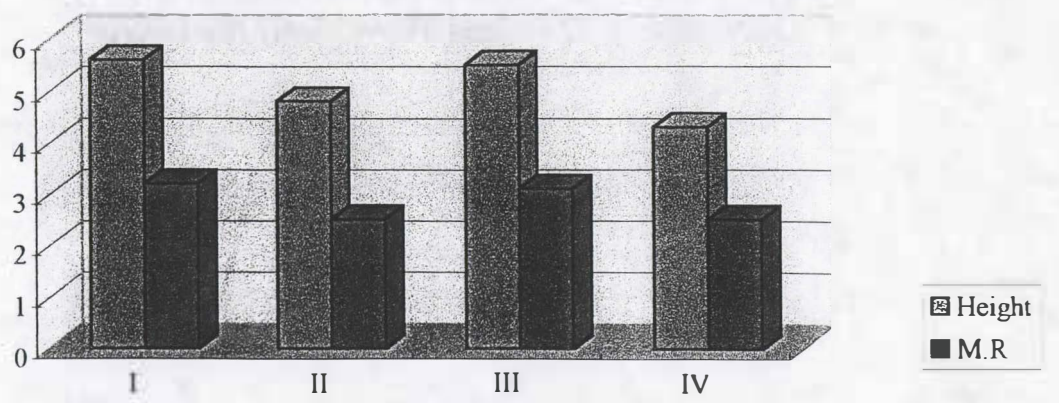
Plant material: Solomon Island clone no.3

Objective: To determine the height and multiplication rate of plantlets in the full and half concentration of iron and nutrients. The experiment has been conducted for 1 year consisting of transfer at 6-week intervals.

Treatment: (I) Full concentration of Iron and nutrients, alternate with or without BA.
(II) Half concentration of Iron and nutrients, alternate with or without BA.
(III) Full concentration of nutrients and half concentration of iron, alternate with or without BA.
(IV) Half concentrationof nutrients and full concentration of iron, alternate with or without BA.

Treatment	I	II	III	IV
Height	5.6	4.8	5.5	4.3
M.R	3.2	2.5	3.1	2.5

Height and Multiplication Rate of Clone no.3 in Full and Half Concentration of Iron and nutrients



Results: (1) Were observed a higher multiplication rate and average height in both full and half concentration of iron as well as nutrients.

(2) There is a direct correlation between the height and multiplication rate i.e taller plantlets give higher multiplication rate.

Conclusion: Preliminary result indicates that the concentration of iron has no influence on the growth rate, but, the concentration of nutrients did; higher multiplication rate and height were obtained.

- *Experiment 5 : **Effect of different concentrations of hormones on shoot growth of different teak clones***

The main objective of this experiment is to optimize the current conditions of *in vitro* propagation of teak in order to improve the acclimatization success. Although this acclimatization success was shown to reach 95% of the plantlets with juvenile material in the best conditions of weaning, this percentage remains unknown from adult material. In addition, as we have noticed that the non-rooted plantlets were slower to be acclimatized than the rooted ones, the development of improved culture media could enhance the percentage of rooting obtained at the end of the last *in vitro* culture cycle just before the *ex vitro* transfer. This percentage of non-rooted plantlets is relatively high since it represents about half of the total number.
Some of the clonal materials used in this experiment will also be used in a next clonal test in comparison with clonal materials from other origins not tested yet in the field and for regeneration and replacement of the stock plants in nursery.

- Plant material

Seven selected clones from Kota Marudu : clones no. TG 20, TG 22, TG 23, TG 25, TG 32, TG 45 and TG 47 (Sabah origin), two clones from Solomon Islands (TG 49 and TG 51) and two from Kampung Apas (TG 13 and TG 16) were used in this experiment. The material from Kota Marudu originates from "plus" trees selected by PISP in 1996 while clones from the two other origins were unselected materials use as control treatments. After collecting shoots and twigs from the mother trees, they were transferred to sand beds under the misting system in Luasong nursery. Cuttings were prepared from the new developed shoots and the rooted shoots were transferred to polybags. They were stored as stock plants in nursery afterwards. The shoots introduced in this experiment were collected from sprouts of these stock plants.

- *In vitro* culture conditions

Two collections of plant material were done on October 23 and November 7 (see Annex 4 for details). Shoots with one to three nodes were sectionned from the stock plants and introduced *in vitro* the following day. An average of twelve nodal segments per clone were collected in each collection. After a disinfection treatment by immersing the entire shoots in a 0.1% solution of mercuric chloride, single nodal explants were isolated and transferred onto a same introduction medium. Two months later, all explants were transfer onto multiplication medium containing the same basal medium as in the previous introduction medium (usual basal medium for teak) but containing three increasing concentrations of growth regulators : levels 1, 2 and 3. Concentration 3 was 10 times higher than concentration 1 and 2 times higher than concentration 2.
The effect of the three concentrations of growth regulators on shoot growth was analyzed after two months of culture in the first multiplication subculture which followed a two-month culture in the introduction medium at concentration 3. The number of axillary shoots per explant, the mean height and number of nodes per shoot were recorded after two months of culture in the first multiplication subculture.

- Results and discussion

As shown in the table below, the number of axillary shoots produced after two months of culture in multiplication medium were not significantly different according to the hormone concentrations 1 and 2 and between clones. No significant difference was also found in mean shoot height and number of nodes per shoot. By contrast, there was a significant effect of the hormone concentration 3 on shoot growth of clones TG 13, 16 and 20 as we found higher numbers of axillary shoots per explant and lower shoot heights and number of nodes per explant compared to those observed at concentrations 1 and 2. However, shoot growth of the bulk of clones TG 22, 23, 26, 45, 47 at concentration 3 was not significantly different with that obtained in all clones at concentrations 1 and 2.

Effect of three concentrations of hormones added to the multiplication medium on shoot growth of different teak clones after two months of culture :

Hormone concentration *	clone number **	Number of explants tested	no. of axillary shoots/explant	Mean shoot height (mm)	Mean no. of nodes/shoot
1	TG 13	5	1.33	24.5	3.7
	TG 16	5	1.40	35.7	3.1
	TG 20	5	1.20	29.2	2.7
	TG 22, 26, 32, 45	5	1.60	26.3	2.2
2	TG 13	5	1.33	23.2	1.4
	TG 16	7	1.43	28.0	3.3
	TG 20	5	1.40	19.3	2.4
	TG 22, 23, 26, 32, 45, 47	7	1.57	27.3	1.8
3	TG 13	5	3.00	15.0	1.0
	TG 16	6	2.33	24.6	1.8
	TG 20	5	2.33	10.7	2.0
	TG 22, 23, 26, 45, 47	5	1.40	38.6	3.3

* Concentration 3 was 10 times higher than concentration 1 and 2 times higher than concentration 2.
** TG x, y, z,... corresponds to a bulk of the corresponding clones with one to two explants per clone.

As early as just after the introduction stage, axillary branching could be stimulated by a too high concentration of hormones in the medium. A higher production of axillary shoots induced the development of shorter shoots with a lower number of nodes per shoot. However, only three clones exhibited such a response whereas the other clones were less sensitive to a high hormone concentration. The different levels of maturity between the two clonal origins tested could explain the high sensitiveness of clones TG13 and TG 16 observed at concentration 3 since these two clones originate from more juvenile material (Solomon Islands) than the other clones from Kota Marudu (TG 20, 22, 23, 26, 32, 45 and 47) issuing from mature trees. However, TG 20 was highly responsive even though it originates from Kota Marudu.

- Conclusion and perspectives

This current experiment will be carried on for several multiplication subcultures in order to observe the long-term effect of different concentrations of hormones in the multiplication medium on shoot development in comparison with the standard protocol used for teak. Plantlets from the different media will be transferred to polybags in nursery for further observations on the root system and shoot development.

3.2 Rattan species

In this reporting period, all research work on the three species of rattans was halted except for the transfer of somatic plantlets of *C. merrillii* to the nursery for acclimatisation and the introduction of about 300 *C. subinermis* fruits for *in vitro* culture manipulations.

Out of 37 somatic plantlets transferred, only 6 survived and were then transferred to polybags. These plants are now hardening in the nursery in Luasong and will be planted in the field once a suitable height is reached.

To pursue the work on the somatic embryogenesis of *C. subinermis* in particular, fruits were collected from the plots in LFC in July, during the fruiting season. The fruits were cleaned and the embryos were excised and treated as previously described in past steering reports. Each batch of fifty embryos was then inoculated on auxin-containing media containing Picloram at concentrations ranging from 1 to 5 mg/l, and on basal MS medium which acted as a control.

Several weeks after this inoculation, no definite pattern of callogenesis was observed from any of these explants. Only spongy tissue giving evidence of the process was produced; these tissues however failed to develop any further. After a few months, a number of these explants were discarded due to of phenolic oxidation or contamination by fungi.

This result, as that in the past experiment, further confirmed the difficulty of using zygotic embryo as a source of explant for callogenesis unlike for rotan Palasan. As reported then, only roots of C. subinermis appeared to be more responsive for the production of calli in contrast to young leaves or zygotes. Similarly, this holds true for rotan Manau. Only for rotan Palasan was calli easily obtained from zygotic embryos which subsequently developed up to the plantlet stage. Depending upon the availability of plant material, time and manpower, the work on somatic embryogenesis of rotan batu will be continued as a number of questions remain unaswered.

In relation to this work, Dr. Doreen Goh undertook four weeks of training at the CIRAD-BIOTROP laboratory, in Montpellier, France, in April last year. Under the guidance of Dr. Nicole Micaux-Ferriere who is a specialist on the somatic embryogenesis of tropical crop species such as coffee, cocoa, and rubber, a histo-cytological analysis of somatic embryos of both rotan Palasan and Batu was done. This study was done to complement the work on rotan Manau which was undertaken earlier. The work on these two other species was along the same line of investigation with the objective to better understand the whole process and to determine if there are any distinct differences in these three species. Further analyses are however required and this work will continue, particularly if new samples of various stages of calli and somatic embryos can be obtained from new introduction of plant materials such as described above.

4. Root symbioses :

Australian *Acacia* species which are leguminous N_2-fixing trees, are generally spontaneously nodulated by rhizobia in their native area and in soils where it has been introduced. These microsymbionts allow these species to grow on nitrogen-deficient soils through direct fixation of atmospheric nitrogen. So far, most of the studies on the *Acacia*-rhizobium symbiosis have been carried out on *A. mangium*. Although *A. mangium* was shown to be nodulated exclusively by *Bradyrhizobium* strains - slow-growing soil bacteria which are known to have a broad host plant spectrum - *A. mangium* is considered as a specific host plant since only a restricted range of *Bradyrhizobium* strains is able to produce efficient N_2-fixing nodules on the root system. Several inoculation field experiments set up in different countries and on different soil types showed a positive effect of the inoculation with certain strains of *Bradyrhizobium* on *A. mangium* growth several years after transplantation to the field. In certain locations (Côte d'Ivoire), it has been shown that about 50% of the nitrogen content in *A. mangium* leaves originated from nitrogen fixation whereas the other half was directly assimilated by the roots from the different sources of nitrogen present into the soil.

The purpose of our studies on this topic is to evaluate the effect of a rhizobial inoculation on tree growth in nursery conditions and in the field.

- <u>Experiments</u> :

In a first stage, fourteen rhizobium strains from *Acacia mangium x auriculiformis* hybrids and seven from *A. mangium* were isolated from nodules collected in Luasong (see the list of the different strains, their origins and dates of isolation in the table below).

In a second stage, these strains were characterized according to their growth rate on a specific medium in Petri dishes (YEMA medium : Yeast extract mannitol agar medium) and their host plant spectrum. The first part of this host spectrum consisted in inoculating the *A. mangium* and *Acacia* hybrid strains to *A. mangium* and *A. crassicarpa* seedlings grown into tubes under monoxenic conditions. Some collection strains isolated in different countries from *A. mangium*, *A. auriculiformis*, *Acacia holosericea*, *Acacia albida*, and *Macrotyloma africanum* (annual leguminous species) were compared to the new isolated strains. Some other *A. mangium* collection strains (listed in the table below from Lu 4 to Nlu 5), isolated in Sabah and other locations by Francis Martin (CIRAD volunteer in NTU Singapore) before being stored in the PBL since 1994, were also tested.

List, origins and growth rates of rhizobium strains available from the Plant Biotechnology Laboratory (Tawau) :

Strain no.	Host species	Origin	Date of isolation	Growth rate
AH 8c	*A. mangium x auriculiformis* hybrid	Clone 8 (Luasong)	September 1997	Fast-growing
AH 8g	"	"	"	"
AH 8h1	"	"	"	"
AH 8h2	"	"	"	"
AH 8i	"	"	"	"
AH 8j	"	"	"	"
AH 8k	"	"	"	"
AH 10	"	Clone 10 (Luasong)	"	"
AH 11a	"	Clone 11 (Luasong)	"	"
AH 11c	"	"	"	"
AH 11f	"	"	"	"
AH 12c	"	Clone 12 (Luasong)	"	"
AH 17-2	"	Clone 17 (Luasong)	"	"
AH 17-2	"	"	"	"
Am 5-1	*A. mangium*	Luasong (clone no.5,	February 1998	Slow-growing
Am 5-2	"	PNG seed orchard)	"	"
Am 5-3	"	"	"	"
Am 5-4	"	"	"	"
Am 5-5	"	"	"	"
Am 5-6	"	"	"	"
Am 5-7	"	"	"	"
Lu 4	*A.mangium*	Luasong	1994	Slow-growing
Lu 7	"	"	"	"
Nlu 2	"	Luasong (nursery)	"	"
Nlu 3	"	"	"	"
Nlu 5	"	"	"	"
Was 1	"	Taliwas	"	"
Was 2	"	"	"	"
Was 3	"	"	"	"
Was 9	"	"	"	"
Tel 1	"	Telupid	"	"
Tel 2	"	"	"	"
Tel 5	"	"	"	"
Tel 6	"	"	"	"
Tel 8	"	"	"	"
Tel 10	"	"	"	"
But 1	"	Bukit Timah, Singapore	"	"
But 3	"	"	"	"
But 5	"	"	"	"

- Results and discussion :

As reported in the table above, all strains isolated from *A. mangium* were slow-growing rhizobia (= *Bradyrhizobium*) whereas strains from *Acacia mangium x auriculiformis* were fast-growing rhizobia (= *Rhizobium stricto sensus*). These data are particularly interesting since all strains from *A. mangium* isolated throughout the world (refer to the large collection of *A. mangium* strains in LSTM of CIRAD-Forêt) were also shown to be slow-growing rhizobia, as well as the rhizobium strains isolated from *A. auriculiformis*. Surprinsingly, despite the *A. mangium* parental origin of the hybrids, they were spontaneously nodulated in soil by fast-growing rhizobia. Our *in vitro* inoculation experiments showed that all five *Acacia* hybrid strains tested, as well as the other *A. mangium* strains, produced efficient N_2-fixing nodules on *A. mangium* and *A. crassicarpa* (data not reported in details here).

- Conclusion and perspectives :

These data showing that the *Acacia* hybrids are exclusively nodulated by fast-growing rhizobia need to be confirmed in further isolation experiments carried out in various locations and soil types. The fast-growing *Acacia* hybrid rhizobium strains seem to have a large host plant spectrum whereas most of the fast-growing strains from other species are very specific and only nodulate a restricted number of host plants. To our knowledge, this type of fast-growing strain having a large host spectrum was only reported in the *Leucaena leucocephala*-rhizobium association with the well-studied strain NGR234 isolated in Nigeria (Africa).

These *Acacia* hybrid rhizobium strains are currently under genetical characterization through the use of molecular biology methods (PCR/RFLP technique) in a collaboration study with LSTM (Laboratory on Mediterranean and Tropical Symbioses / CIRAD-Forêt Montpellier) that was initiated in the framework of the Hannah MOO's training in France in November 1998. The host plant spectrum of the hybrid strains will be complemented soon in inoculating *A. mangium x A. auriculiformis* hybrid clones and *A. auriculiformis* seedlings.

An inoculation trial was set up in the Brumas nursery (SSSB) in july 1998. In this experiment, three different *Acacia* hybrid clones (acclimatized *in vitro* plantlets from PBL) were inoculated with two strains of rhizobium, one *Acacia* hybrid strain and one *A. mangium* strain. The two inoculation treatments were compared with control treatments corresponding to uninoculated plants. The data will be analyzed soon.

5. COMMERCIALISATION

The consignments to Maju Aik Sdn. Bhd. and RISDA in Peninsular Malaysia were fulfilled with the delivery of 10,000 and 2,000 plantlets, respectively, during March and April. These orders were described in the last steering report. Having met these orders, the decision to reduce the further multiplication of teak was made by the management in the face of reduced market demands. A few hundred plants consisting of both Perlis and Solomon Island origins were subsequently culled in the lab in order to lower the number of plants being maintained.

Toward the last quarter of the year, following a series of exhibitions in which the laboratory participated in a bid to promote the sales of teak at the local level, orders began to flow in once again. As a result, there was a need to step up the multiplication of the two origins which in turn resulted in the significant investment of time and effort by the staff to quickly meet these orders. This of course resulted in a shift in priority of the other ongoing activities in the project. As such, the question of whether the commercialisation activity should be taken on a more official note, especially if the demands have to be met at very short notice, became an issue again.

It was decided by the ICSB management then that production of both tissue cultured-plantlets and macro-cutttings from the nurseries in Taliwas and LFC be stepped up to fulfil the orders that came in. The largest order to date is that of KL Kepong which put in a request of 34,000 plantlets through the PBL. This order will be fulfilled in four batches starting at the end of April to the end of August. In the meantime, both the lab and the nursery at Taliwas have been catering to smaller orders ranging from 3,000 to 10,000 plants from companies like JC Chang, Pahang Oil Palm Estate, and Gerola Estate.

5.1 Sales of Plantlets

In this reporting period, no direct sale of pre-acclimatised tissue cultured-plantlets was made as there was no order for this type of material. As such, overseas buyers so far consisted of only Maju Aik Sdn. Bhd. and RISDA. As described previously, tissue-cultured plantlets were acclimatised in the nursery in Taliwas and were sold only when they were at the ready-for-planting stage. For the local markets, although it may be more economical to produce rooted cuttings than micro-cuttings, it is noteworthy that the production of the micro-shoots is less time-consuming and certainly more reliable than production at the nursery. As such, despite orders made at short notice, the PBL staff have been doing an excellent job of meeting orders in as short a time period as possible. Too many factors in the nursery such as the responsiveness of cuttings, the skilfulness of the personnel doing the manipulation, and the conditions of the stock plants, may influence a consistent above average rooting rate, and thus the final output.

5.2 Service Contracts

Since the signing of the agreements for the two service contracts, the past year had been a busy time for the staff particularly in fulfilling the service contract with SSSB. The multiplication of *Acacia* spp., particularly, *Acacia* hybrids, went into full swing following the successful introduction of a total of 27 clones collected on four different occasions. In addition, there was also a few collections and subsequent multiplication of *A. crassicarpa* (See Annex).

For teak, although the stabilisation phase took from 3 to 6 months before the multiplication phase was possible, manipulation of these materials was done very carefully. This was particularly important after the proposed trip to Mata Ayer for the third collection of materials was postponed indefinitely until further notice in light of the budget woes for ICSB. An update on the status of these two contracts is given in the subsequent sections.

5.2.1. ICSB-FRIM

This service contract between FRIM and ICSB was officially agreed on February 1, 1998. The contract was on the micropropagation of teak clones selected jointly by both FRIM and ICSB/CIRAD-Foret researchers. The ortets are located in several research plots in Mata Ayer, Perlis. A description of the first collection trip undertaken in early December, 1997, was given in the Steering Committee report last year. For information on the current status of the clones collected, refer to the next two pages.

5.2.2. ICSB-SSSB

As described earlier, the introduction of clones of *Acacia* hybrids *and A. crassicarpa* from the field and nursery in Brumas, SSSB, was done on a regular basis over the past year. A summary of the status of this service contract is given in the pages after the summary for the ICSB-FRIM agreement.

ICSB-FRIM AGREEMENT

Mata Ayer Clones Introduction

This is a brief update on the status of the clones collected on two occasions from the FRIM research station in Mata Ayer. The first collection was done in December 1997 while the second collection was in May 1998.

On the first trip, 17 clones were selected. The trees at this time of the year were observed to be going through the dormancy period as indicated by the absence of new or young branches. The tips of most shoots were bud-like, and the internodes were shorter and more woody in appearance. In contrast, on the second collection in May, which was soon after the flushing period, new shoots were seen on most trees. At this time, a total of 22 clones was selected. Of these 22 clones, ten were already collected on the first trip but were again collected due to their failure to respond the first time (except for T11). Altogether, the introduction of 27 clones from both collections was attempted (see Table)

Out of these 27 clones, only 7 have been successfully introduced and stabilised under the given tissue culture conditions. It should be noted that most explants reached the stabilisation phase after 3 to 6 months from the initial introduction phase. Regular transfers to prevent mortality due to contamination and phenolic oxidation were required. Both endogenous bacterial d fungal contamination were particularly rampant in explants from the second collection. As a result, most explants were eventually discarded.

The Table also shows the 7 clones which are successfully stabilised and multiplied. As seen, 5 out of 17 and 3 out of 22 clones from the first and second collections, respectively, are now undergoing the multiplication phase. The highest number of plants multiplied is 231 from clone T10 and the lowest is 9 from clone T5. The oldest ortet successfully introduced is B98, planted in 1953 from Burma origin. It should be noted that explants from all these 7 clones, though in the multiplication phase, do not show vigorous growth or development. The plantlets are quite small in size with short internodes. Also, multiple axillary shoots (3 to 4) are seen arising from a number of plantlets which may contribute to the slow growth of these plants. Overall, the multiplication rate ranges from 1 to 2 among these clones at an interval of 8 weeks per cycle.

ICSB-FRIM AGREEMENT
MATA AYER CLONES INTRODUCTION

Introd. Dec 1997 ID No.	Introd. May 1998 ID. No.	Year Planted/Origin	[1]No. multiplied 1st + 2nd Coll.	[2]Present No.
A4	A4-	1962, Jeniang-P. Malaysia	0	
-	A20	1968, Trinidad Island	0	
B1	B1	1953, Burma	0	
B3	B3	1962, P. M'sia	1+0	
	B46	1967, Burma	0	
	B73	1968, Trinidad Island	0	
	B79	1968, Trinidad Is.	0	
	B80	1968, Trinidad Is.	0	
	B93	1953, India	1	
	B94	1953, India	0	
B97	B97	1953, Burma	0	
B98	B98	1953, Burma	0+2	10
B106	-	1979, Provenance unknown	4+0	162
T2	-	1980, India	13+0	89
-	T4	1980, India	0	
T5	-	1980, India	4	12
T7	T7	1982, India	0	
T10	-	1980, India	123	240
T11	T11	1982, India	3 + 2	78
T17	T17	1982, India	0	
-	T18	1982, India	0	
T19	T19	1982, India	0	
-	T32	1980, India	8	80
-	T34a	1983, India	0	
-	T34b	1983, India	0	
T47	-	1967, Burma	0	
1962-1	1962-1	1962, P. M'sia	1	
17 clones	22 clones			671

1 Count as of August 1998
2 Count as of March 1999

5.2.2. ICSB-SSSB

A service contract was concluded between ICSB and Sabah Softwoods Sdn. Bhd. on January 15, 1998 for the micropropagation of clonal materials of *Acacia* hybrids and *A. crassicarpa* selected by SSSB. 20,000 plantlets of these species were requested in this agreement, the deliveries of the plantlets being expected in the first quarter of 1999. These plantlets will be used further by SSSB as stock plants in a multiplication clonal garden for the mass production of cuttings.

The *in vitro* introduction and production of the SSSB clonal materials started at the end of January 1998 for *Acacia* hybrids and end of april 1998 for *Acacia crassicarpa*. The different dates of *in vitro* introduction, multiplication and rooting subcultures carried out from the different plant sources are listed in the tables of Annexes no.1 and 3.

These clones consist of 30 *A. mangium x auriculiformis* hybrid and 20 *A. crassicarpa* genotypes. The hybrids clones were already selected by SSSB whereas the *A. crassicarpa* clones were selected jointly by the PISP, PBL and SSSB in April 1998.

5.2.2.1. Plant material

1) - *Acacia mangium x auriculiformis* hybrids

a) *Acacia* hybrid clones from Ulu Kukut, Brumas and Silam :

The shoots to be introduced were collected from young cuttings consisting of 16 different clones currently maintained in Brumas nursery : clones no. 1 to 16 (except no. 12 that was not available) and clone 483/34, the clone numbers having been assigned by SSSB.

b) *Acacia* hybrid clones from the SSSB 1993 clonal test :

These clones correspond to 15 superior trees. The shoots were collected from stock plants originating from marcots. The clone numbers assigned by SSSB are as follows AA17F105, AA7D15, AA7F110, AM7C9, AM7C3, AA7D16, AA7D6, AA7F112, AA7F113, AA7D18, AA7F117, AA7D12, AA7D17, AA7F72 and AA7F45. These clones were renamed by PBL after *in vitro* introduction as clone numbers S17 to S 30 respectively. So far, 13 out of 15 initial clones from the SSSB 1993 clonal test are still under multiplication (clones no. AA17F105 and AA7F117 were lost).

2) *Acacia crassicarpa* :

A first stage of data assessment, selection and identification of the 20 best individuals among the best families (in terms of volume growth and bole straightness more especially) was performed in the SSSB 1995 progeny trial in Brumas in collaboration with the SSSB, PBL and PISP teams. Twenty "plus" trees were selected within the five best families out of 62 families

(see the localization of each clone within the experimental design in the table below). Outperforming individuals from other families were also selected from the 6th to 10th best ranked families but have not been yet collected. In a second stage, shoots were directly collected from tree branches of the selected "plus" trees for *in vitro* introduction. At the same time, marcots from the selected "plus" trees were prepared by the SSSB team for further clonal propagation through cuttings.

Origins of the *Acacia crassicarpa* plus trees selected from the 1995 Progeny Test, Brumas (SSSB) :

Block	Treatment no.	Tree no.	Clone no.*
1	15	8	CR1
1	14	2	CR2
1	35	7	CR3
2	23	2	CR4
3	19	8	CR5
3	23	8	CR6
3	15	10	CR7
4	23	6	CR8
4	14	6	CR9
4	14	9	CR10
4	14	10	CR11
1	19	5	CR12
2	51	4	CR13
2	35	3	CR14
2	46	1	CR15
2	46	7	CR16
2	52	10	CR17
2	28	7	CR18
3	25	10	CR19
3	9	5	CR20

* The initials CR sometimes attached to the *A. crassicarpa* clone numbers and assigned by the PBL correspond to the initials PT assigned by SSSB.

Due to the very low success of introduction in *A. crassicarpa*, four successive batches of introduction were needed to get a sufficient number of responsive and contaminant-free explants for further multiplication. These collections occured from the end of April up to December 1998, the two last ones having been performed from marcots (see table, Annex 1 p.66). This poor introduction success, especially in the two first batches, was due to high levels of fungal contaminations and a poor responsiveness of the introduced nodes as shown by table of Annex 1.

5.2.2.2. Culture cycles and dates of transfer :

A table attached in Annex 11 (p.88) sums up the sequence of subcultures from the introduction phase up to the rooting phase (for more details, see the three related progress reports). In this paragraph were just updated the last unreported transfer operations.

A) Introduction stage :

The day following shoot collection, single-node segments were excised from the shoots and introduced *in vitro* into individual tubes after a disinfection treatment.

- *A. crassicarpa* clones from the SSSB 1995 progeny test :

- In the first introduction (April 24, 1998), the shoots were collected from trees no. CR1 to CR 11 with a mean number of 48 introduced nodes per clone. Only 1% out of 523 initial explants responded positively against 21% with no reaction and 78% contaminated by fungal infections.
- In the second introduction (June 6, 1998), 33 to 40 single nodal explants from 14 trees (CR2, CR7, CR9, and CR10 to CR20) were introduced *in vitro* but again, only 2% out of 526 explants responded positively against 22% with no reaction and 76% contaminated by fungi.
- In the third introduction (July 11, 1998), a mean number of about 27 single nodal explants from 11 plus tree clones (CR1 to CR11) were collected. They originated from the rooted marcots recently transferred from the 1995 Progeny trial to the SSSB Research nursery. With 8% of responsiveness out of 292 explants, the introduction success was low but higher than in the 2 first collections.
- In the fourth introduction (December 16, 1998), 20 clones (CR1 to CR20) were collected from marcots. A mean number of about 16 explants per clone were introduced. In this last batch, 22% of explants were successfully introduced.

B) Multiplication cycles :

1) *Acacia mangium x auriculiformis* hybrids

As calculated from the tables of Annex 11 (p.88), we obtained an average multiplication rate of 2.1 every 6 weeks (mean periodicity per multiplication subculture) all batches and clones combined, corresponding to a monthly multiplication rate of 2.9. However, this multiplication rate was only obtained 5 to 6 months after introduction as it is shown by the exponential production curves for each batch in Annex 12 (p.89).

2) *A. crassicarpa* clones from the SSSB 1995 progeny test :

At the end of February 1999, 516 plantlets from the three first introduction batches were under multiplication medium and 204 from the fourth one, giving a total of 720 plantlets all together. These plantlets are still under multiplication and will be transferred onto

multiplication medium for the last time in April before transferring them onto rooting medium. 17 out of 20 initial clones are represented, clones CR 1, CR 3 and CR 15 having been lost by infections. However, the plantlets from clones CR6 and CR7 represent 75% of the total number of plantlets at the moment.

C) Rooting phase :

1) - *Acacia mangium x auriculiformis* hybrids

a) *Acacia* hybrid clones from Ulu Kukut, Brumas and Silam :

In January, a number of 8980 plantlets was obtained from the sixteen clones after division and transfer of all multiplied shoots onto rooting medium (see Annex 11, p.88). This number corresponded to 66% of the total number of *Acacia* hybrid plantlets (13,580) that was transferred onto rooting medium until early February.

b) *Acacia* hybrid clones from the SSSB 1993 clonal test :

In early February, 4600 plantlets from the thirteen clones were transferred onto rooting medium. This amount corresponded to 34% of the total number of *Acacia* hybrid plantlets available at the same moment.

- Conclusion :

From the 13,575 *Acacia* hybrid plantlets that were transferred onto rooting medium in mid-February, 8,500 plantlets are now rooted. Since at least five weeks are required for the development and division of the new rooted shoots, we have reached a constant monthly production of 4,000 plantlets. Thus, four batches of 4,000 plantlets are expected to be delivered to SSSB (Brumas nursery) from April to July. 3,000 *A. crassicarpa* plantlets and 1,000 additional *Acacia* hybrids will be delivered in July to reach the 20,000 required.

6. PUBLICATIONS, SCIENTIFIC CONTACTS AND COMMON PROJECTS

6.1. Publications

As in the past, for a better recognition of our activities, there is a continued need to publish in International journals. A list of high level publications is quite useful and even necessary especially when applying for scientific projects with financial support such as the two EU-funded STD3 projects.

The list of publications produced by the Plant Biotech Unit team since the beginning is as given in Annex 8.

6.2. Scientific contacts, training, collaborations and common projects:

Besides the international scientific contacts of the project leaders for the ultimate benefits of the Plant Biotech Lab, participation in two international scientific projects, from which financial supports have been generously obtained, are as described:

- E.E.C. STD III: Genetic resources and improvement of South-East Asia rattan species, with the support of electrophoresis investigations. This project started on January 1st, 1995 and was officially extended until June 1998.

As highlighted earlier, histo-cytological investigations on somatic embryogenesis of C. subinermis and C. merrillii were carried out in mid-April, 1998, in the CIRAD-BIOTROP laboratories in Montpellier by Dr Doreen GOH, as agreed during the last STD III meeting held in February 1997 in Kuala Lumpur. Following this 3-week training, the final meeting for this project occurred in May in Kuala Lumpur. At this meeting, all participants of the project presented their studies undertaken during the past three years. The final report was submitted in June and a review of these reports is being compiled and will be published.

- E.E.C. STD III: Biotechnology applied to *Acacia mangium*, with a view for genetic engineering. In the same way as for the E.E.C. STD III Rattan Project, this project was also accepted by E.E.C. to be funded for 3 years. This project started on October 1st, 1994 and was extended to April 1, 1998. The final report of this project was submitted before June last year. A review of the work done on the transformation of *Acacia* species will be published in relation to the studies done on *A. mangium*.

6.2.1. Final and progress reports :

GALIANA A. FRANCHE C., LIMANTON A., GRATIO V., AHEE J. and E. DUHOUX, 1998. Regeneration and genetic transformation in Australian Acacias, 16 p. Scientific final report (31/3/1998). ERBTS3 contract from the European Union 1994-1997 : "Improving the growth of tropical nitrogen-fixing forest trees in the genera *Acacia* and *Casuarina* through tissue culture and genetic transformation".

GALIANA A., 1998. Report of a mission to Singapore from 16 to 19 march, 1998, in the framework of the BIORIZE Project : "Assessment of the effect of Biorize Endomycorrhizal inoculants on *Acacia mangium* growth and performance", 12 p.

GALIANA A. and D. GOH, 1998. First quarterly report on the progress of *in vitro* production of *A. crassicarpa* and *Acacia mangium x auriculiformis* hybrid clones from Sabah Softwoods Sdn. Bhd. carried out by the Plant Biotechnology Laboratory (ICSB/CIRAD-Forêt, Tawau), September-November 1998, 7 p.

GALIANA A. and D. GOH, 1998. Second quarterly progress report of *in vitro* production of *Acacia mangium x auriculiformis* hybrid and *A. crassicarpa* clones from Sabah Softwoods Sdn. Bhd. carried out by the Plant Biotechnology Laboratory (ICSB/CIRAD-Forêt, Tawau), May-July 1998, 7 p.

GALIANA A. and D. GOH, 1998. Third quarterly report on the *in vitro* production of *Acacia mangium x auriculiformis* hybrid and *A. crassicarpa* clones from Sabah Softwoods Sdn. Bhd. carried out by the Plant Biotechnology Laboratory (ICSB/CIRAD-Forêt, Tawau), September-November 1998, 7 p.

GOH, D.K.S. Scientific final report (31/3/1998). ERBTS3*CT940278 contract from the EuropeanUnion 1994-1998 : "Improving the growth of tropical nitrogen-fixing forest trees in the genera *Acacia* and *Casuarina* through tissue culture and genetic transformation".

GOH, D.K.S. Scientific final report (9/1998). ERTS3*CT94-0285 contract from the European Union 1994-1998: "Conservtion, Genetic Improvement and Silviculture of Rattan species in South East Asia".

- Nyanyang Technological University / Singapore : Scientific collaboration with Pr. LEE SING KONG) and scientific support to Laurent GAUTRY (volunteer in Singapore founded by the French Foreign Ministry) in the framework of the CIRAD/NTU Project completed in 1998 : "Rehabilitation of degraded lands through aeroponic mass propagation of clones of some nitrogen-fixing tree species selected for optimum symbiotic association" (Cf mission report, 1998) ;

- GeneTrop/ORSTOM Laboratory, Montpellier : Participation to the STD 3 Project : "Improving the growth of tropical nitrogen-fixing trees in the genera *Acacia* and *Casuarina* through tissue culture and genetic transformation" : Completion of the Project in 1998 (final report) and writing of publications related to tissue culture and genetic transformation of Australian Acacias.

- Back-up missions to Nyanyang Technological University (Singapore) : Scientific collaboration with Pr. LEE SING KONG (setting up of experiments in greenhouse) and scientific support to Laurent GAUTRY CSN/MAE in the framework of the Project founded by the French Foreign Ministry (MAE) : "Rehabilitation of degraded lands through aeroponic mass propagation of clones of some nitrogen-fixing tree species selected for optimum symbiotic association".

- Mission to peninsular Malaysia with FRIM (State of Perlis) from 17 to 20 May 1998 : Joint mission ICSB/FRIM/CIRAD-Forêt : Second collection of twigs and shoots on "plus" trees selected by FRIM in FRIM and Forestry Departement's plantations (Mata Ayer) for further *in vitro* introduction and multiplication in Plant Biotechnology Laboratory.

6.2.2. Trainings :

A two week training undertaken by Doreen GOH at the CIRAD-BIOTROP laboratory, Montpellier, FRANCE, in April 1998 on the "Histo-cytological Analysis of Somatic Embryos of *Calamus meriillii* and *C. subinermis*" under the guidance of Dr. N. M-Ferriere. Funding was provided under the framework of the EU-funded STD3 Project on"Conservtion, Genetic Improvement and Silviculture of Rattan species in South East Asia".

Participation of Antoine GALIANA to a training course organized by AGETROP (Genetic Laboratory CIRAD-Montpellier/France) from 14 to 22 September 1998 : "Using of molecular markers in genetics and plant

In the framework of the MOU between ICSB and CIRAD-Foret, staff training of ICSB staff is provided by Cirad-Foret as in the past. Hanna Moo undertook a 6-week training at the Genetic Laboratory, CIRAD-Foret and at the LSTM (Tropical and Mediterranean Symbiosis Laboratory- CIRAD/ORSTOM/INRA Common Project) in Montpellier, France, under the guidance of Dr. M. Chevallier and Dr. Y. Prin, respectively. Her training involved the genetic analyses of Acacia species using isozymes and DNA, as well as a short study on various *Rhizobial* species.

7. SPECIFIC QUESTIONS AND PERSPECTIVES

1. This year about 40,000 teak plantlets were transferred from the lab to the nurseries for much needed field trials in Taliwas and LFC and for meeting the orders from local buyers. The capability of the PBL to mass produce this species particularly with commercialisation in mind is going smoothly and has so far been successful in meeting demands of buyers at short notice. Although it may not be very profitable (unless orders are large) to produce tissue-cultured plants for sales in local markets for the long term, it is undeniable that the reliability and amount of time saved from using this method are highly attractive. This is particularly true in term of meeting orders quickly.

2. The structural deterioration within the laboratory over the past year once again highlights the problem of whether there is a need to renovate the establishment to prevent further degradation or look at other alternatives. As reported earlier, there is now an obvious crack between the ground and first floor of the building caused by the shifting of the ground. As a precaution, we were obliged to move certain equipment away from this area in case of falling debris from the ceiling. In relation to this is the problem of space as highlighted in the last report. The micropropagation of both teak and Acacia spp. are taking up a big part of the space in the two main culture rooms, resulting in the conversion of the dark room to a lighted one. As such, experiments requiring complete darkness have been reduced to avoid the light problems. However, more important is the issue of the production capacity of the lab if there is a need to meet large orders within a short span of time. The question of whether this is possible in relation to the constraint in space remains in view of the other ongoing activities on research.

INTRODUCTION OF *ACACIA CRASSICARPA* SHOOTS
FOR IN-VITRO CULTURE FROM SABAH SOFTWOODS, BRUMAS
-SERVICE CONTRACT

CLONE ID	DATE INTROD.	NO. INTROD.	NO. FUNGI CONT	NO. BACT. CONT	NO. DEAD	NO. IN STABLN PHASE	% OF SUCCESS. INTRODN
CR 1	24.04.98	48	33	4	11	0	0.0
CR 2	24.04.98	44	40	2	2	0	0.0
CR 2	04.06.98	33	24	4	5	0	0.0
CR 3	24.04.98	48	41	4	3	0	0.0
CR 4	24.04.98	48	43	1	4	0	0.0
CR 5	24.04.98	48	33	3	10	2	4.2
CR 6	24.04.98	47	35	1	11	0	0.0
CR 7	24.04.98	48	46	2	0	0	0.0
CR 7	04.06.98	33	24	0	7	2	6.1
CR 8	24.04.98	48	36	2	10	0	0.0
CR 9	24.04.98	48	29	0	19	0	0.0
CR 9	04.06.98	33	16	1	13	3	9.1
CR 10	24.04.98	48	39	1	8	0	0.0
CR 10	04.06.98	33	22	2	8	1	3.0
CR 11	24.04.98	48	36	3	9	0	0.0
CR 11	04.06.98	34	26	1	7	0	0.0
CR 12	04.06.98	40	30	2	8	0	0.0
CR 13	04.06.98	40	31	1	8	0	0.0
CR 14	04.06.98	40	35	2	2	1	2.5
CR 15	04.06.98	40	30	2	8	0	0.0
CR 16	04.06.98	40	39	1	0	0	0.0
CR 17	04.06.98	40	30	0	10	0	0.0
CR 18	04.06.98	40	32	0	8	0	0.0
CR 19	04.06.98	40	30	2	8	0	0.0
CR 20	04.06.98	40	20	3	16	1	0.0

** The clones CR 1 to 20 are the same as PT 1 to 20.

- CONTINUATION -

CLONE ID.	DATE INTROD.	NO. INTROD	NO. FUNGI CONT	NO. BACT CONT	NO. DEAD	NO. IN STABILN PHASE	% OF SUCCESS. INTRODN
PT 1	11.07.98	7	6	0	1	0	0.0
PT 1	06.08.98	48	26	9	9	4	8.3
PT 2	11.07.98	29	13	5	8	3	10.3
PT 2	06.08.98	77	28	19	20	10	13.0
PT 2	16.12.98	16	10	1	4	1	6.3
PT 3	11.07.98	7	5	1	1	0	0.0
PT 3	06.08.98	98	58	24	16	2	2.0
PT 4	11.07.98	39	19	9	11	0	0.0
PT 4	06.08.98	80	3	1	76	0	0.0
PT 4	16.12.98	16	1	3	8	4	25.0
PT 5	11.07.98	33	19	5	9	1	3.0
PT 5	06.08.98	80	29	16	21	14	17.5
PT 5	16.12.98	16	5	3	7	1	6.3
PT 6	11.07.98	38	23	5	10	4	10.5
PT 6	06.08.98	80	48	18	14	0	0.0
PT 6	16.12.98	16	8	0	2	6	37.5
PT 7	11.07.98	13	7	1	1	4	30.8
PT 7	06.08.98	103	32	19	33	19	18.4
PT 7	16.12.98	16	5	0	1	10	62.5
PT 8	11.07.98	64	39	11	13	1	1.6
PT 8	06.08.98	80	44	19	15	2	2.5
PT 8	16.12.98	16	6	1	2	7	43.8
PT 9	11.07.98	49	21	12	16	2	4.1
PT 9	06.08.98	80	26	12	18	24	30.0
PT 9	16.12.98	16	5	2	6	3	18.8
PT 10 & PT 11	11.07.98	13	5	2	1	5	38.5
PT 10	06.08.98	35	21	3	7	4	11.4
PT 10	16.12.98	16	1	2	5	8	50.0
PT 11	06.08.98	48	28	5	7	8	16.7
PT 11	16.12.98	15	4	1	2	8	53.3

- CONTINUATION -

CLONE ID.	DATE INTROD.	NO. INTROD.	NO. FUNGI CONT	NO. BACT. CONT	NO. DEAD	NO. IN STABILN PHASE	% OF SUCCESS INTROD.
PT 12	16.12.98	21	7	3	7	4	19.0
PT 14	16.12.98	24	7	4	6	7	29.2
PT 16	16.12.98	19	4	5	8	2	10.5
PT 17	16.12.98	24	9	4	9	2	8.3
PT 18	16.12.98	24	8	3	6	7	29.2
PT 19	06.08.98	95	55	18	18	4	4.2
PT 19	16.12.98	24	11	3	6	4	16.7
PT 20	16.12.98	22	4	6	9	3	13.6
PT 131*	16.12.98	11	3	1	4	3	27.3
PT 132*	16.12.98	24	4	5	14	1	4.2

**PT 131 and 132 - Identities to be further confirmed

INTRODUCTION OF *A. CRASSICARPA* SHOOTS FROM 10-YEAR OLD ORTETS, PNG ORIGIN SEED STAND, LUASONG FORESTRY CENTER

CLONE ID.	DATE INTROD.	NO. INTROD.	NO. FUNGI CONT	NO. BACT. CONT	NO. DEAD	NO. IN STABILN PHASE	% OF SUCCESS INTROD.
AC 2	04.06.98	89	42	10	9	28	31.5
AC 3	23.07.98	191	107	25	59	42	22.0
AC 4	23.07.98	102	66	16	15	5	4.9
AC 11	25.06.98	103	49	17	12	25	24.3
AC 29	25.06.98	36	18	11	7	0	0.0
AC 36	23.07.98	109	58	18	13	27	24.8
AC 37	04.06.98	150	91	31	21	7	4.7
AC 44	25.06.98	87	33	17	19	18	20.7
AC 45	23.07.98	149	56	21	28	44	29.5

INTRODUCTION OF *A. MANGIUM* SHOOTS FROM 10-YEAR OLD ORTET, PNG ORIGIN SEED STAND, LUASONG FORESTRY CENTER

CLONE NO.	DATE INTROD.	NO. INTROD	NO. FUNGI CONT	NO. BACT. CONT	NO. DEAD	NO. IN STABILN PHASE	% OF SUCCESS INTROD.
AM 6	04.06.98	206	125	33	47	1	0.5
AM 6	25.06.98	246	137	51	57	1	0.4

INTRODUCTION OF *ACACIA* HYBRIDS SHOOTS FOR *IN-VITRO* CULTURE FROM SABAH SOFTWOODS, BRUMAS -SERVICE CONTRACT

CLONE ID.	DATE INTROD.	NO. INTROD.	NO. FUNGI CONT	NO. BACT. CONT	NO. DEAD	NO. IN STABILN HASE	% OF SUCCESS INTROD.
S 17	07.03.98	41	9	16	0	16	39.0
S 17	25.04.98	24	8	5	8	3	12.5
S 18	07.03.98	48	16	5	0	28	58.3
S 18	25.04.98	24	6	3	8	7	29.2
S 19	07.03.98	48	33	2	0	13	27.1
S 19	25.04.98	24	7	2	7	8	33.3
S 20	07.03.98	48	25	11	0	12	25.0
S 20	25.04.98	39	10	3	12	14	35.9
S 21	07.03.98	48	13	6	0	29	60.4
S 21	25.04.98	38	17	4	8	9	23.7
S 22	07.03.98	48	17	7	8	16	33.3
S 22	25.04.98	16	6	2	5	3	18.8
S 23	07.03.98	44	16	3	12	13	29.5
S 23	25.04.98	24	14	3	6	1	4.2
S 24	07.03.98	32	14	10	0	8	25.0
S 24	25.04.98	21	7	3	8	3	14.3
S 25	07.03.98	48	27	10	0	11	22.9
S 25	25.04.98	24	12	2	8	2	8.3
S 26	25.04.98	19	7	2	6	4	21.0
S 27	07.03.98	48	17	2	3	26	54.2
S 27	25.04.98	24	10	4	8	2	8.3
S 28	07.03.98	48	32	1	0	15	31.2
S 28	25.04.98	24	11	2	5	6	25.0
S 29	07.03.98	36	16	7	0	13	36.1
S 29	25.04.98	16	5	4	2	5	31.3
S 30	07.03.98	46	17	3	10	16	34.8
S 30	25.04.98	24	12	4	6	2	8.3
AA7F112	07.03.98	48	36	0	1	11	22.9
7 C9	25.04.98	24	5	5	5	9	37.5

SUCCESS RATE OF TEAK SHOOTS INTRODUCTION FOR TISSUE CULTURE FROM FEB 1998 TO MARCH 1999 FROM THE LUASONG FORESTRY CENTER AND TALIWAS * NURSERIES

CLONE NO.	DATE INTROD	NO. INTROD	NO. FUNG CONT	NO. BACT CONT	NO.OF UNRESP. EXPLTS[1]	NO. IN STABILN PHASE[2]	% OF SUCCESS INTROD.
1	13.8.98	19	11	6	2	0	0.0
1*	17.9.98	6	6	0	0	0	0.0
1*	16.10.98	31	22	5	4	0	0.0
TOTAL		56	39	11	6	0	0.0
2	13.8.98	18	12	3	3	0	0.0
2	10.9.98	68	44	17	7	0	0.0
2*	17.9.98	6	5	0	1	0	0.0
2	24.9.98	32	20	7	4	1	3.1
2*	16.10.98	16	13	3	0	0	0.0
TOTAL		140	94	30	15	1	0.7
3	13.08.98	16	10	2	1	3	18.8
3	10.09.98	17	13	0	4	0	0.0
TOTAL		33	23	2	5	3	9.1
4	13.08.98	18	11	3	2	2	11.1
4	10.09.98	27	17	6	4	0	0.0
4*	17.09.98	10	5	4	0	1	10.0
4	24.09.98	56	32	17	7	0	0.0
4*	16.10.98	23	16	4	1	2	8.7
TOTAL		134	81	34	14	5	3.7
5*	17.09.98	8	7	1	0	0	0.0
5	24.09.98	33	25	5	3	0	0.0
5*	16.10.98	35	28	5	2	0	0.0
TOTAL		76	60	11	5	0	0.0

- CONTINUATION -

CLONE NO.	DATE OF INTROD	NO. INTROD	NO. FUNG CONT	NO. BACT CONT	NO. OF UNRESP EXPLNT[1]	NO. IN STABILN PHASE[2]	% OF SUCCESS INTROD
6	13.08.98	11	8	2	1	0	0.0
6	20.08.98	20	11	6	3	0	0.0
6	28.08.98	70	62	7	1	0	0.0
6	10.09.98	37	29	6	2	0	0.0
6*	17.09.98	6	5	0	1	0	0.0
6	24.09.98	24	15	5	4	0	0.0
6*	16.10.98	24	17	6	1	0	0.0
6	11.11.98	18	11	1	3	3	16.5
6	14.01.99	38	16	5	17	0	0.0
TOTAL		248	174	38	33	3	1.2
7*	17.09.98	10	5	0	1	4	40.0
7*	16.10.98	33	28	4	1	0	0.0
TOTAL		43	33	4	2	4	9.3
8	24.09.98	47	35	7	5	0	0.0
10	14.01.99	23	13	3	3	4	17.3
13	23.10.98	26	7	1	1	17	65.4
13	07.11.98	22	6	3	3	10	45.5
TOTAL		48	13	4	4	27	56.3
16	23.10.98	13	3	0	0	10	76.9
16	07.11.98	9	3	0	1	5	55.6
TOTAL		22	6	0	1	15	68.2
20	23.10.98	48	10	2	2	34	70.8
20	07.11.98	28	5	1	2	20	71.4
20	11.12.98	45	19	4	4	18	40.0
TOTAL		121	34	7	8	72	59.5

- CONTINUATION -

CLONE NO.	DATE OF INTROD	NO. INTROD	NO. FUNG CONT	NO. BACT CONT	NO. OF UNRESP EXPLNT[1]	NO. IN STABILN PHASE[2]	% OF SUCCESS INTROD.
22	23.10.98	8	0	0	0	8	100.0
22	07.11.98	10	2	0	0	8	80.0
22	11.12.98	12	8	1	1	2	16.7
TOTAL		30	10	1	1	18	60.0
23	23.10.98	3	0	0	0	3	100.0
23	07.11.98	3	2	0	0	1	33.3
23	11.12.98	6	3	0	0	3	50.0
TOTAL		12	5	0	0	7	58.3
25	23.10.98	4	2	0	1	1	25.0
25	07.11.98	10	4	1	1	4	40.0
25	11.12.98	15	8	1	2	4	26.7
TOTAL		29	14	2	4	9	31.0
26	07.11.98	2	0	0	0	2	100.0
32	23.10.98	6	3	1	0	2	33.3
32	07.11.98	6	5	0	1	0	0.0
32	11.12.98	10	9	0	1	0	0.0
TOTAL		22	17	1	2	2	9.1
45	23.10.98	4	1	0	0	3	75.0
45	07.11.98	7	3	0	1	3	42.9
TOTAL		11	4	0	1	6	54.5
47	23.10.98	2	0	0	0	2	100.0
47	07.11.98	4	0	0	0	4	100.0
TOTAL		6	0	0	0	6	100.0
49	23.10.98	2	1	0	0	1	50.0
51	23.10.98	26	14	3	2	7	26.9
51	07.11.98	16	8	3	1	4	25.0
TOTAL		42	22	6	3	11	26.2

- CONTINUATION -

CLONE NO.	DATE OF INTROD.	NO. INTROD	NO. FUNG CONT	NO. BACT CONT	NO. OF UNRESP EXPLNT[1]	NO. IN STABILN PHASE[2]	% OF SUCCESS INTROD.
PERLIS BULK	13.08.98	66	40	20	6	0	0.0
"	20.08.98	210	130	51	29	0	0.0
"	28.08.98	227	154	52	20	1	0.4
"	10.09.98	224	173	29	19	3	1.3
"	24.09.98	179	142	27	10	0	0.0
"	08.10.98	280	207	37	35	1	0.4
"	11.11.98	103	77	20	6	0	0.0
"	11.12.98	115	112	1	0	2	1.7
"	14.01.99	118	91	4	10	13	11.0
TOTAL		1522	1126	241	135	20	1.3
SOL. IS BULK	24.09.98	120	87	23	10	0	0.0
" *	17.12.98	78	52	8	4	14	18.0
" *	08.01.99	105	81	12	3	9	8.6
" *	05.02.99	80	30	3	7	40	50.0
TOTAL		383	250	46	24	63	16.4

1 refers to explants which did not respond to *in vitro* conditions

2 refers to explants which are successfully introduced and stabilised for subsequent multiplication

Table I: Number of Teak Tissue Culture Plantlets sent to LFC Nursery for Acclimatization

DATE TRANSFER	PLANT ORIGIN	NO. TRANSFER	SUBTOTAL
15-7-1998	Solomon Island clone no.9 (progeny)	300	
	Solomon Island I	45	
	Solomon Island II	44	
	Solomon Island clone no.3	47	
	Kg. Apas clone no.16	46	482
22-7-1998	Kota Marudu clone no.29	22	
	Kota Marudu clone no.44	50	72
3-3-1999	Perlis Bulk	1,000	1,000
18-3-1999	Solomon Island clone no.3	1,537	
	Solomon Island clone no.8	201	1,738
25-3-1999	Solomon Island mericlone no.3	1,190	1,190
		Total	**4,482**

Table II: Number of Teak Tissue Culture plantlets sent to Taliwas Nursery for Acclimatization

DATE TRANSFER	PLANT ORIGIN	NO. TRANSFER	SUBTOTAL
7-3-1998	Kg. Apas clone no.16	1,782	
	Kota Marudu clone no.21	54	
	Kota Marudu clone no.25	540	
	Kota Marudu clone no.42	104	
	M-1	353	
	M-12	48	
	M-13	879	
	Solomon Island – Bulk	51	3,811
18-5-1998	Solomon Island – Bulk (meristem culture source)	2,700	
	Solomon Island clone no.3	1,740	
	Perlis – Bulk	1,500	5,940
24-6-1998	Solomon Island clone no.5	150	
	Kota Marudu clone no.21	190	
	Kota Marudu clone no.22	300	
	Kota Marudu clone no.25	95	
	Kota Marudu clone no.27	25	
	Kota Marudu clone no.41	85	
	Kota Marudu clone no.42	200	

(continue) Table II

DATE TRANSFER	PLANT ORIGIN	NO. TRANSFER	SUBTOTAL
	Thailand I – Bulk	210	
	Thailand II – Bulk	40	
	India I – Bulk	100	
	Kota Marudu – Bulk	135	1,530
3-7-1998	Solomon Island II	1,000	1,000
21-7-1998	Solomon Island clone no.3	32	
	Kg. Apas clone no.16	104	
	Solomon Island clone no.9 (progeny)	732	
	Solomon Island I	620	
	Solomon Island II	119	
	Solomon Island – Bulk (meristem culture source)	510	
	Perlis – Bulk	266	2,455
8-8-1998	Solomon Island – Bulk	1,034	
	Solomon Island clone no.3	1,466	2,500
15-9-1998	Solomon Island – Bulk	2,424	
	Perlis – Bulk	12	2,436
18-11-1998	Solomon Island clone no.3 (meristem culture source)	1,028	1,028
16-12-1998	Solomon Island – Bulk	1,217	
	Solomon Island clone no.3	30	1,520
7-1-1999	Solomon Island clone no.3 (meristem culture source)	1,700	
	Solomon Island clone no.3	302	
	Solomon Island – Bulk	40	
	Perlis Bulk	20	2,062
4-2-1999	Solomon Island – Bulk	3,014	3,014
19-2-1999	Perlis Bulk	120	120
25-2-1999	Perlis Bulk	2,360	
	Solomon Island clone no.3	120	2,479
5-3-1999	Perlis Bulk	127	
	Solomon Island clone no.3	117	244
23-3-1999	Solomon Island – Bulk	3,013	3,013
		TOTAL	**33,279**

GROWTH DATA FROM *IN VITRO* CULTURES OF
SHOOT APICAL MERISTEMS OF CLONES FROM SOLOMON ISLAND ORIGIN
FOR REPORTING PERIOD FEB 1998 TO MARCH 1999

MERI-CLONE/ LINES	DATE OF INTROD.	NO. INTROD	AVERAGE HEIGHT OF PRIMARY SHOOT (CM)*	MULT. RATE*	NO. MAINT. FOR DATA COLLECTN
1	3-12-96	11	3.1	2.0	37
3-A	3-12-96	1	4.7	2.8	30
3-B	"	1	5.4	3.2	30
3-C	"	1	4.6	3.0	30
3-D	"	1	3.9	2.7	30
3-E	"	1	4.8	2.7	30
3-F	"	1	5.2	3.1	30
3-G	"	1	4.2	2.7	30
3-H	"	1	5.0	3.1	30
3-I	"	1	5.2	3.0	10
3-J	"	1	4.7	3.5	10
3-K	"	1	5.2	3.0	10
3-L	"	1	6.1	3.7	10
3-M	"	1	5.1	3.1	10
3-N	"	1	4.1	2.7	10
3-O	"	1	4.4	3.1	10
3	14-1-97	22	2.8	1.7	42
5	14-1-97	22	3.8	2.0	40
7	14-1-97	26	2.1	1.3	NA**
8	14-1-97	10	2.5	1.7	43

* VALUES REFLECT THE AVERAGES OF DATA COLLECTED AT EVERY TRANSFER SINCE THE INITIAL INTRODUCTION OF EXPLANTS

** REMAINING EXPLANT WAS DISPLACED WITHOUT TRACE

Tissue-Cultured Teak Plantlets used for Commercial Sales

DATE TRANSFER	PLANT ORIGIN	NO. TRANSFER	SUBTOTAL
7-3-1998	Solomon Island -- Bulk	51	51
18-5-1998	Solomon Island – Bulk (meristem culture source)	2,700	
	Solomon Island clone no.3	1,740	
	Perlis –Bulk	1,500	5,940
15-7-1998	Solomon Island clone no.3	46	46
21-7-1998	Solomon Island clone no.3	32	
	Solomon Island – Bulk (meristem culture source)	510	
	Perlis -- Bulk	266	808
8-8-1998	Solomon Island – Bulk	1,034	
	Solomon Island clone no.3	1,466	2,500
15-9-1998	Solomon Island – Bulk	2,424	
	Perlis -- Bulk	12	2,436
18-11-1998	Solomon Island clone no.3 (meristem culture source)	1,028	1,028
16-12-1998	Solomon Island – Bulk	1,217	
	Solomon Island clone no.3	303	1,520
7-1-1999	Solomon Island clone no.3 (meristem culture source)	1,700	
	Solomon Island clone no.3	302	
	Solomon Island – Bulk	40	
	Perlis -- Bulk	20	2,062
4-2-1999	Solomon Island -- Bulk	3,014	3,014
19-2-1999	Perlis -- Bulk	120	120
25-2-1999	Perlis – Bulk	2,360	
	Solomon Island clone no.3	120	2,479
5-3-1999	Perlis – Bulk	127	
	Solomon Island clone no.3	117	244
18-3-1999	Solomon Island clone no.3	1,537	1,537
23-3-1999	Solomon Island -- Bulk	3,013	3,013
25-3-1999	Solomon Island mericlone no.3	1,190	1,190
		Total	**27,988**

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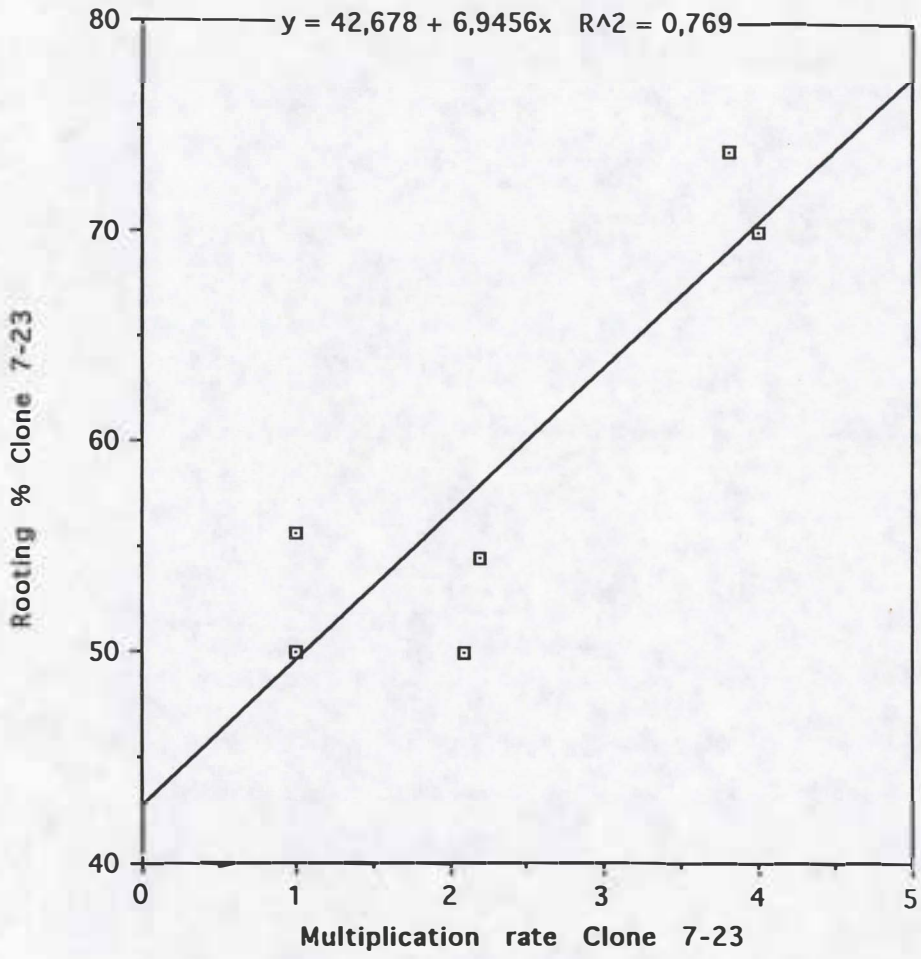

y = 42,678 + 6,9456x R^2 = 0,769
Rooting % Clone 7-23
80
70
60
50
40
0
1
2
3
4
5
Multiplication rate Clone 7-23

Acacia hybrids

Experiment 1 : Effects of clone and auxin concentration on rooting % of 2-month-old shoots 7 weeks after transfer onto rooting medium

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Clone	2	4275,83	2137,92	5,26	,0092
Auxin concentration	2	2642,32	1321,16	3,25	,0488
Clone * Auxin concentration	4	2265,98	566,49	1,39	,2527
Residual	41	16651,25	406,13		

Dependent: % rooting 7 weeks

Means Table
Effect: Clone * Auxin concentration
Dependent: % rooting 7 weeks

	Count	Mean	Std. Dev.	Std. Error
-21, 0,2	5	48,00	17,89	8,00
-21, 0,5	5	58,00	16,43	7,35
-21, 1,0	5	64,66	29,60	13,24
-13, 0,2	6	19,85	16,84	6,88
-13, 0,5	6	56,67	25,82	10,54
-13, 1,0	6	39,07	13,52	5,52
-23, 0,2	5	58,00	13,04	5,83
-23, 0,5	6	65,00	20,74	8,47
-23, 1,0	6	52,60	21,40	8,74

Means Table
Effect: Clone
Dependent: % rooting 7 weeks

	Count	Mean	Std. Dev.	Std. Error
-21	15	56,89	21,66	5,59
-13	18	38,53	23,93	5,64
-23	17	58,56	18,68	4,53

Student-Newman-Keuls
Effect: Clone
Dependent: % rooting 7 weeks
Significance level: ,05

	Count	Mean		
5-13	18	38,53	a	
3-21	15	56,89		b
7-23	17	58,56		b

Means Table
Effect: Auxin concentration
Dependent: % rooting 7 weeks

	Count	Mean	Std. Dev.	Std. Error
2	16	40,57	22,73	5,68
5	17	60,00	20,62	5,00
0	17	51,37	23,06	5,59

Student-Newman-Keuls
Effect: Auxin concentration
Dependent: % rooting 7 weeks
Significance level: ,05

	Count	Mean		
0,2	16	40,57	a	
1,0	17	51,37	a	b
0,5	17	60,00		b

Annex 10 (continued)

Acacia hybrids

Experiment 3 : Effects of shoot size and type of auxin on rooting % of 2-month-old shoots from clone 5-13, 7 weeks after transfer onto rooting medium

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Type of shoot	1	1056,25	1056,25	5,45	,0478
Type of auxin and concentration	3	4618,75	1539,58	7,95	,0088
Type of shoot * Type of auxin and ...	3	368,75	122,92	,63	,6134
Residual	8	1550,00	193,75		

Dependent: % rooting 7 weeks

Means Table
Effect: Type of shoot * Type of auxin and concentration
Dependent: % rooting 7 weeks

	Count	Mean	Std. Dev.	Std. Error
ong, IAA 0,3	2	40,00	0,00	0,00
ong, IBA 0,2	2	25,00	21,21	15,00
ong, NAA 0,2	2	35,00	7,07	5,00
ong, None	2	70,00	14,14	10,00
hort, IAA 0,3	2	20,00	14,14	10,00
hort, IBA 0,2	2	0,00	0,00	0,00
hort, NAA 0,2	2	35,00	21,21	15,00
hort, None	2	50,00	14,14	10,00

Means Table
Effect: Type of shoot
Dependent: % rooting 7 weeks

	Count	Mean	Std. Dev.	Std. Error
ong	8	42,50	20,53	7,26
hort	8	26,25	22,64	8,00

Student-Newman-Keuls
Effect: Type of shoot
Dependent: % rooting 7 weeks
Significance level: ,05

	Count	Mean	
Short	8	26,25	a
Long	8	42,50	b

All were significantly different at this level.

Means Table
Effect: Type of auxin and concentration
Dependent: % rooting 7 weeks

	Count	Mean	Std. Dev.	Std. Error
A 0,3	4	30,00	14,14	7,07
A 0,2	4	12,50	18,93	9,46
AA 0,2	4	35,00	12,91	6,45
one	4	60,00	16,33	8,16

Student-Newman-Keuls
Effect: Type of auxin and concentration
Dependent: % rooting 7 weeks
Significance level: ,05

	Count	Mean	
IBA 0,2	4	12,50	a
IAA 0,3	4	30,00	a
NAA 0,2	4	35,00	a
None	4	60,00	b

Acacia hybrids

Experiment 4 : Effects of clone and type of auxin on rooting % 6 weeks after transfer onto rooting medium

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Clone	1	282,27	282,27	2,71	,1381
Type of auxin and concentration	1	699,21	699,21	6,72	,0320
Clone * Type of auxin and concentration	1	5,88	5,88	,06	,8181
Residual	8	832,23	104,03		

Dependent: Rooting % 6 weeks

Means Table
Effect: Clone * Type of auxin and concentration
Dependent: Rooting % 6 weeks

	Count	Mean	Std. Dev.	Std. Error
3-21, IAA 0.3	3	44,43	9,64	5,57
3-21, IBA 0.2	3	27,77	9,58	5,53
5-13, IAA 0.3	3	33,33	14,43	8,33
5-13, IBA 0.2	3	19,47	4,79	2,77

Means Table
Effect: Clone
Dependent: Rooting % 6 weeks

	Count	Mean	Std. Dev.	Std. Error
3-21	6	36,10	12,54	5,12
5-13	6	26,40	12,26	5,00

Student-Newman-Keuls
Effect: Clone
Dependent: Rooting % 6 weeks
Significance level: ,05

	Count	Mean	
5-13	6	26,40	a
3-21	6	36,10	a

None were significantly different at this level.

Means Table
Effect: Type of auxin and concentration
Dependent: Rooting % 6 weeks

	Count	Mean	Std. Dev.	Std. Error
IAA 0.3	6	38,88	12,55	5,12
IBA 0.2	6	23,62	8,16	3,33

Student-Newman-Keuls
Effect: Type of auxin and concentration
Dependent: Rooting % 6 weeks
Significance level: ,05

	Count	Mean	
IBA 0.2	6	23,62	a
IAA 0.3	6	38,88	b

All were significantly different at this level.

Acacia hybrids

Experiment 7 : Effects of the gelling agent and medium strength in macroelements on the number of roots/explant in clone 3-21, 2 weeks after transfer onto rooting medium

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Medium strength	1	7,81	7,81	4,43	,0389
Gelling agent	3	16,04	5,35	3,03	,0349
Medium strength * Gelling agent	3	4,04	1,35	,76	,5189
Residual	72	127,10	1,77		

Dependent: Rooting % 2 weeks

Means Table
Effect: Medium strength * Gelling agent
Dependent: Rooting % 2 weeks

	Count	Mean	Std. Dev.	Std. Error
ull strength, Gelan Gum 0.25%	10	,50	,71	,22
ull strength, Gelan Gum 0.3%	10	,60	,97	,31
ull strength, Phytagel 0.2%	10	,50	,97	,31
ull strength, Phytagel 0.3%	10	1,20	1,32	,42
alf strength, Gelan Gum 0.25%	10	1,50	1,35	,43
alf strength, Gelan Gum 0.3%	10	1,20	1,62	,51
alf strength, Phytagel 0.2%	10	,40	,84	,27
alf strength, Phytagel 0.3%	10	2,20	2,20	,70

s Table
t: Medium strength
ident: Rooting % 2 weeks

	Count	Mean	Std. Dev.	Std. Error
rength	40	,70	1,02	,16
trength	40	1,33	1,65	,26

Student-Newman-Keuls
Effect: Medium strength
Dependent: Rooting % 2 weeks
Significance level: ,05

	Count	Mean	
Full strength	40	,70	a
Half strength	40	1,33	b

All were significantly different at this level.

s Table
t: Gelling agent
ident: Rooting % 2 weeks

	Count	Mean	Std. Dev.	Std. Error
Gum 0.25%	20	1,00	1,17	,26
Gum 0.3%	20	,90	1,33	,30
el 0.2%	20	,45	,89	,20
el 0.3%	20	1,70	1,84	,41

Student-Newman-Keuls
Effect: Gelling agent
Dependent: Rooting % 2 weeks
Significance level: ,05

	Count	Mean		
Phytagel 0.2%	20	,45	a	
Gelan Gum 0.3%	20	,90	a	b
Gelan Gum 0.25%	20	1,00	a	b
Phytagel 0.3%	20	1,70		b

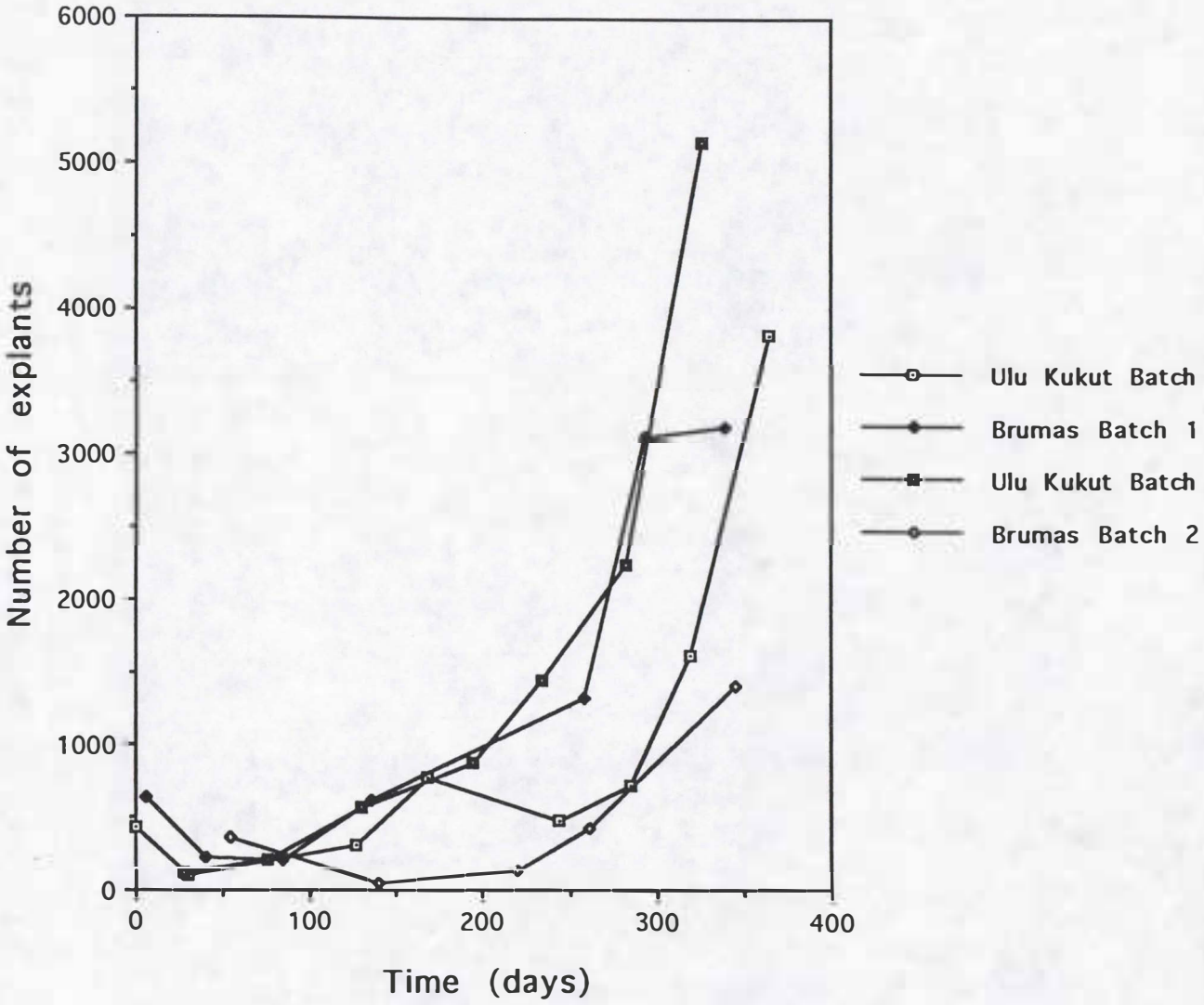
Annex 11 :

***In vitro* production of *Acacia* hybrids selected clones from Ulu Kukut, Brumas and Silam (SSSB clonal collection)**

Culture cycle	Transferring date of batch no. 1	Number of explants transferred	Transferring date of batch no. 2	Number of explants transferred
Introduction	January 29	432	March 27	125
Multiplication 1	February 28	97	May 15	209
Multiplication 2	June 5	312	July 7	576
Multiplication 3	July 16	771	September 10	878
Multiplication 4	September 29	482	October 20	1444
Multiplication 5	November 9	722	December 7	2240
Multiplication 6	December 14	1618	-	-
Rooting	January 28	3836	January 20	5159

***In vitro* production of *Acacia* hybrids selected clones from the 1993 clonal test (SSSB clonal collection)**

Culture cycle	Transferring date of batch no. 1	Number of explants transferred	Transferring date of batch no. 2	Number of explants transferred
Introduction	March 6	631	April 23	365
Multiplication 1	April 9	231	July 17	56
Multiplication 2	May 23	213	October 5	134
Multiplication 3	July 13	613	November 16	429
Multiplication 4	October 1	678	December 28	714
Multiplication 5	November 12	1327	-	-
Multiplication 6	December 18	3128	-	-
Rooting	February 2	3185	February 8	1412


6000
5000
4000
3000
2000
1000
0
Number of explants
0
100
200
300
400
Time (days)
Ulu Kukut Batch
Brumas Batch 1
Ulu Kukut Batch
Brumas Batch 2